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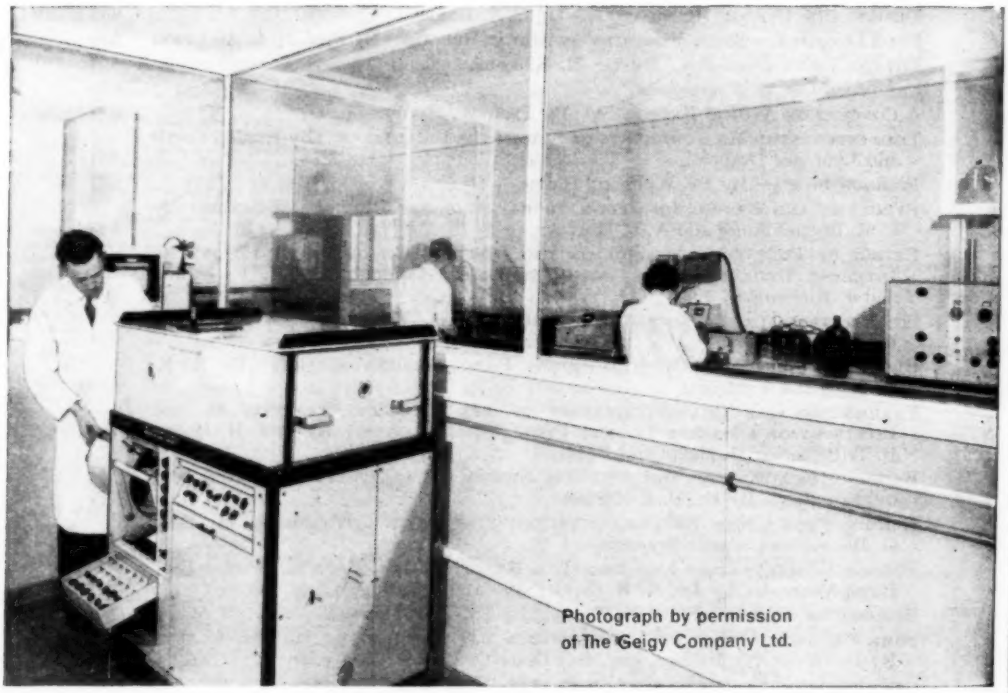

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FURTHER ADVANCEMENTS IN SPACE RESEARCH

COMMENTING on the latest Soviet achievement in space science, last year's president of the British Association for the Advancement of Science, Sir George Thomson, said: "If you have done a thing once it is not difficult to do it several times. It seems to me a cheerful Bank Holiday event, and we should thank the Russians for arranging it". After pondering these remarks they would appear less waggish and more profound than at first. The first remark is, however, rather naïve, for there can be no doubt that this second successful attempt to put man into orbit embraced more scientific 'things', with consequent results from which much can be gleaned, than were inherent in the first successful attempt, with Major Yuri Gagarin in the space-vehicle. The second remark, however, needed making. The news would be cheerful whether the feat was achieved by Soviet scientists, American scientists or those of any other nationality: for here, once again, is the manifestation of great triumphs for scientists, coupled with the heroism of another—this time Major G. S. Titov, of the Soviet Air Force.

The spaceship-satellite *Vostok 2*, 1961 τ, launched from the U.S.S.R. at 06.00 hr. U.T. on August 6, carried the second man to travel in orbit around the Earth. The satellite, which weighed 4,730 kgm. excluding the final-stage rocket, was projected into an orbit inclined at 64° 56′ to the equator and having an orbital period of 88·6 min. Its height above the Earth varied between 178 km. at perigee and 257 km. at apogee. Its radio transmitters operated on frequencies of 15·765, 19·995, 20·006 and 143·625 Mc./s. *Vostok 1*, which carried Major Gagarin in the first manned orbital flight on April 12 (see *Nature*, 190, 307; 1961), made one revolution about the Earth. *Vostok 2* made 17 revolutions, and, since the orbital period was such that the geographical track on the seventeenth revolution was almost identical with that on the first, Major Titov made his descent in the same region as Major Gagarin. Major Titov landed safely near Saratov, some 500 miles south-east of Moscow, after a flight lasting 25 hr. 18 min. The main object of this second orbital flight was to test the effects on a human being of a longer journey in orbit. Major Titov reported that his condition was excellent throughout his epoch-making flight, and the prolonged period of weightlessness appeared to have no ill-effects. He slept for 8 hr. and ate several meals, taking the food from tubes, as well as operating various manual controls in the satellite, observing the Earth from one of the three portholes, and sending messages to the peoples of the countries he passed over.

On this second occasion more details of experimental conditions were revealed, and even some preliminary results announced—an excellent and cheerful omen for scientific advancement on an international scale. Hitherto, far too much lip-service has been paid by Soviet and American political leaders to the ideal of sharing their scientific results; but little has so far accrued.

There can be no gainsaying the fact that the Soviet scientists are in the vanguard of space travel; but it does not follow that they are ahead of others in space research *in toto*. There are many who believe that the U.S.S.R., through their awe-inspiring achievements, are deliberately playing to the political gallery. It is obvious that their successes are enhancing the political prestige of the Soviets throughout the world, especially in scientifically and culturally backward and emergent countries. But it would be wiser for world leaders in policy to look on these successes in space-travel as the result of hard and inspired scientific endeavour made by scientists who are highly respected and encouraged by their political leaders.

Even in their own country, certainly outside it, Gagarin and Titov are presented to the public as great heroes, which they surely are. But it is essential to recognize that the real prime-movers in these fantastic feats are the scientists and technologists who, through their ingenuity and devoted effort, have made such feats possible. The lay public, both Soviet and millions of others, will acclaim the heroism of men such as Gagarin and Titov; but world leaders would be better advised to take heed of the words of Prof. Nikolay Barabashov, who said: "The general triumph of Soviet *scientists and engineers* consists in that they have created a spaceship controlled by man".

There are still too many otherwise intelligent people who grudgingly now admit that we have had science thrust upon us. They will continue that attitude at their peril, for this is the Age of Science, and, like it or not, we must do all we can to give moral, and especially practical, support to our scientists (in all branches). This is best done by learning and teaching more about science, for then shall we treat scientists for what they really are—world leaders in their own sphere, not 'backroom boys'.

Walter Bagehot said that: "Governments are only strong when public opinion is definable and decided". The strength of democratic Governments must now be enhanced by the support of a public opinion inspired by knowledge of, and greater respect for, science and scientists.

NATIONAL ORGANIZATION OF SCIENTIFIC RESEARCH

THE last report of the Council for Scientific and Industrial Research stressed the growing importance of the Council's influence on university research. This necessarily arises out of the Council's concern with the balance of research within and between the scientific disciplines, and its effort, at the request of the Advisory Council on Scientific Policy, to bridge any important research gaps which come to light and thus promote a healthy balance of research effort. It also arises as a counterpart of the Department's expanding programme of research grants and training awards, which are bound to affect university developments quite apart from the attempts of the Council to encourage co-operative effort, as, for example, in grants for the provision of nuclear reactors for training and research or by specia encouragement for research in certain fringe subjects, such as biophysics, geophysics, meteorology and oceanography, where the Council found insufficient research effort in the country.

Generally, the Council regards the balance of research effort as reasonable in the main streams of development within the chief scientific disciplines, though, to keep this effort adequate and to develop it flexibly, substantial and increasing resources must be given to research through existing channels. The Council is already prepared to make grants for longer periods than hitherto and help initiate projects which for one reason or another seem to require its specially extended support; but nevertheless, when university researches are well established, the Council considers that they are best supported from normal university funds, and the Council welcomes the action of the University Grants Committee in making arrangements for the universities to seek funds for the new quinquennium 1962–67, to continue researches which have been established with the aid of grants from the Department of Scientific and Industrial Research. The Council has already invited the universities concerned to consider whether they should seek funds from the University Grants Committee to carry on most of the research programmes receiving current grants and is discussing these arrangements with the universities.

In this situation, a statement on "Scientific Progress, the Universities and the Federal Government", issued last November in the United States by the President's Science Advisory Committee, to which Dr. Glenn T. Seaborg, chairman of the United States Atomic Energy Commission, made some reference in addressing the American Physical Society on April 26, on atomic energy research, is of particular interest. Dr. Seaborg did not discuss the Commission's programme of applied research; his address was rather on basic research in physical science, and before discussing the Commission's activities in basic research he quoted freely from the Science Advisory Committee's statement to illustrate his own fundamental philosophy of basic science.

The statement, which deals essentially with the problems arising in the advancement of science by basic research and the graduate education of scientists, stresses first the urgent need for scientific progress and for investment in science to that end, without, however, either overlooking the returns such investment also yields in the quality and humanity of our investigation or suggesting that scientific research and scientific education are the only kinds of learning that matter. It affirms unequivocably that basic research and graduate education go best together at every possible level, and equally it insists on the disappearance of the barriers between pure and applied science. Basic research depends in the end on the imaginative powers and scientific skill of its practitioners, and partly because of this the apprentice scientist, even if his subsequent career were not attained in basic research, learns best in an atmosphere of creative research with the depth of understanding of a subject that only comes with such experience.

The President's Science Advisory Committee is under no illusions that this always obtains in the universities or in the Federal agencies, but it suggests that if a research field is to attract able young scientists, it generally needs roots in the universities. A scientific field which has no research life in the universities is at a grave disadvantage in recruiting new talent, nor can research be sustained without teaching, and it is always important for research institutions to maintain effective connexions with students. Not all basic research should or could be pursued in universities, but where it is done elsewhere the Committee believes that special efforts should be made to take advantage of its educational value.

The Committee thus came to consider the role of the Federal Government, which by 1957–58 was providing 70 per cent of the funds for research in American universities, three-quarters of which came from either health or defence agencies. The use of research contracts and research grants is then appraised, and, in spite of the high quality of much research, particularly in nuclear physics, carried out under Government contracts, the Committee regards the research grant, on balance and in the long run, as the better instrument for basic research. It points out, however, that the support of research projects does not, by itself, fully meet the needs of the Federal Government, and that no narrow or single instrument or method of action will serve the Government's purpose.

This appears particularly in considering the fields of science which are comparatively neglected and for which it is difficult for the universities to find funds

or facilities. Like the Advisory Council for Scientific Policy and the Council for Scientific and Industrial Research in Britain, it suggests that such areas of scientific promise call for action by the central Government in initiative and support, and one such area instanced is oceanography. Nevertheless, the statement suggests that the greatest interest of the central Government is to see that university science is as strong as possible, and whether basic research and graduate education in the United States are adequate in quantity and quality depends primarily on the Government: either the Federal Government finds the policies and resources which permit the universities to flourish and discharge their duties adequately, or no one will.

The Committee recognizes that the American universities have themselves much to do and that they have been slow in finding effective ways of encouraging scientific research and training at all the new levels and in all the new ways that scientific advance makes possible. Graduate education is not so good as it should be, and new fields of study are ignored because they inconveniently cross departmental barriers. The Committee does not, however, share the view that Government support is necessarily subversive of academic freedom, though it recognizes its dangers; but it suggests that the most important single task of the universities is to maintain their own standards of freedom and excellence in this period of growing dependence on the State. In the United States, as in Britain, the main trouble in the universities is neither failure of understanding nor communication, but simply lack of means, and it is from this similar context that the recommendations of the statement derive their special interest in Britain at this juncture.

Some specific conclusions should, however, be noted; first, not only on account of their relevance to the general organization of scientific effort, but also equally for their bearing on the expansion of the universities and the colleges of technology. Stress is laid first on the support of excellence. It is of the first importance that national support for both research and teaching should aim at sustaining and reinforcing outstanding work wherever it may be found. Examination of the implications of this conclusion could go far to resolve some recent controversies, and it should be noted that the President's Science Advisory Committee also considers that additional centres of excellence are urgently needed, that graduate education should be expanded and include a genuine experience of research, and that it is important to attract a larger number of talented students to science as a career. Graduate education in science itself needs constant modernizing, and its financing should be continuously and flexibly reinforced, particularly by fellowships.

From what has already been noted it might be expected that the Committee would conclude that new fields of research and education need particular encouragement and that separate research institutions should be avoided wherever possible It is emphatic that in the absence of special considerations the university is the best place for basic research and concludes that new laboratories for special fields should be attached to the universities whenever it is practicable, and that universities should make full educational use of such facilities. It is interesting to note the Committee's opinion that scientists outside the universities can play a fruitful part in graduate education, and it is not only in the United States that there are opportunities demanding exploration and exploitation by the Government, by industry, by universities, and by colleges of technology.

Of the Committee's recommendations, some six were selected by Dr. Seaborg to express his own philosophy on basic research. First, there is the general responsibility. "In view of the growing importance of scientific research to National security and welfare, all parts of the National community should assume a greater responsibility for supporting, strengthening and expanding basic research in graduate education." Secondly, turning to Government responsibility, "Federal support for basic research and graduate education in the sciences should be continued and flexibly increased so as to support excellence where it already exists and to encourage new centres of outstanding work". Thirdly, "The Federal Government should continue and enlarge the practice now followed with great success in a few agencies of providing research support over long terms and for broad objectives". Fourthly, "The Government should strengthen its ability to establish general policies governing its support of basic research and graduate education at the universities". Fifthly, as regards the universities, "Universities should accept primary responsibility for ensuring that their growing partnership with the Government reinforces their freedom and excellence", and sixthly, and generally, "It should be a general basis of policy and action that basic research and the education of scientists go best together, that they are inseparable functions of the Universities".

These recommendations are cited by Dr. Seaborg to illustrate his own approach to basic research, an approach which has not been modified by his experience as chairman of the Atomic Energy Commission. He does not suggest that these are the only important recommendations of the report, but it is interesting to note how largely the Advisory Council of Scientific Policy in Britain appears to be guided by similar views. Nevertheless, the connexion between research and teaching is emphasized in a much more forthright manner than has been apparent in recent discussions in the United Kingdom, and the stress laid on excellence gives no support for the mediocrity and triviality which Sir Eric Ashby and others have criticized.

If this report, therefore, contains much that is of interest to the University Grants Committee and the Robbins Committee also, as well as to the Ministry for Science, Dr. Seaborg's examination of the policies and programmes of the Atomic Energy Commission in the light of these findings is of particular interest in the context of adjustment of Britain's scientific effort with which the Advisory Council on Scientific

Policy is concerned. Dr. Seaborg points out that the budget of the National Science Foundation has already been increased by some sixty-five million dollars to implement some of these recommendations, and two-thirds of this is for support of basic research in the sciences and to improve research facilities. Further, he stresses the close co-operation and exchange of information that already exist between the Commission and the other four Federal agencies that disburse about 90 per cent of Federal funds for basic research. There is some reason for concern as to whether that liaison is sufficiently effective in Britain, and this may be one reason for the misgivings that have been openly expressed as to the deployment of our scientific man-power. At the Baird Memorial Lecture in Glasgow, in May, Dr. S. C. Curran suggested that scientific advance in some fields was hamstrung by the excessive effort being made in such fields as nuclear energy, for example, and on the other hand, the existing relations between the Atomic Energy Authority and the Central Electricity Generating Board appear to offer at least some possibility of duplication of effort. It is by no means clear that the Board's new nuclear laboratory on which £1 million is to be spent, with a staff of 250–500, can do anything which could not be done with the Authority's existing plant and research teams. While there are advantages in the decentralization of nuclear research and engineering, they will not be realized without a careful review of organization and some paring of the effort for which the Authority has hitherto been receiving a consulting fee of £300,000 from the Board.

Dr. Seaborg's remarks about the Atomic Energy Commission's relations with the National Science Foundation and with the universities are of special interest at the present time. Under an agreement with the Foundation, the Foundation takes care of the Commission's projects which involve support of university research and training reactors, but while the universities are part of the foundation on which the United States nuclear energy programme is built, the pattern of arrangements varies widely. At Brookhaven National Laboratory, for example, which is managed for the Commission by Associated Universities, Inc., some 170 scientists are engaged in research and about 300 more from university faculties spend considerable time each year at the Laboratory on research projects. The Argonne National Laboratory is operated under a contract with the University of Chicago, but the thirty-one constituent schools of Associated Mid-West Universities play a vital part in the Laboratory's programme with more than 100 postgraduate research workers, while the Laboratory also operates the International Institute for Nuclear Science and Engineering. Again, at the Lawrence Radiation Laboratory in the University of California, Berkeley, where about 250 graduate workers are employed, basic research is undertaken by faculty members on a part-time basis and by full-time non-academic personnel. Again, at the Oak Ridge National Laboratory, the Union Carbide Nuclear Co. co-operates in making the facilities available to universities, for example, through the Oak Ridge Institution of Nuclear Studies, comprising 39 universities in the south.

Dr. Seaborg gives some figures to indicate the extent of the Commission's research effort. At the end of the past fiscal year, the Commission had more than 900 contracts totalling more than thirty-six million dollars for unclassified basic research in the physical and biological sciences with some 162 educational institutions. Some 330 schools have been assisted with grants totalling 18·5 million dollars, and loans of nuclear materials valued at eleven million dollars and about 1,000 fellowships have been awarded. Basic research supported by the Commission in the physical sciences is mainly in physics and mathematics (including high- and low-energy nuclear physics, computor research and mathematics), metallurgy and related sciences; chemistry and controlled thermonuclear research.

Dr. Seaborg referred to the way in which the Commission is seeking to strengthen research in the metallurgy and material sciences throughout the country, and also to the existence of areas where imaginative contributions of the highest creative type were needed to advance the country's welfare not simply in the nuclear energy programme but in such fields as the long-range transmission of electric power, long-range prediction of weather, the desalting of sea-water, and the application of biophysics and biochemistry to the whole problem of living matter. He was prepared to consider allowing the Commission's National Laboratories to contribute to the solution of such problems even if this went beyond their present functions.

The choice of method or organization is bound to be influenced by the objective or subject of a research project and that alone is sufficient reason for requiring variety and flexibility in the organization of research, whether fundamental or applied. While the points made by the President's Science Advisory Committee and by Dr. Seaborg are not new, they direct attention to first principles to which full regard should be paid alike by the University Grants Committee in considering the expansion of university education and by the Advisory Council on Scientific Policy and the Council for Scientific and Industrial Research in guiding the deployment of Britain's research effort. Furthermore, the extent to which that expansion and research effort are interlocked points to the importance of some co-ordinating authority at a higher level, if frustrations or waste are not to occur. Both through its research grants and its post-graduate training awards the Department of Scientific and Industrial Research cannot now help strongly influencing university development or even policy, however carefully it may seek to avoid infringing academic influences. For example, after the Department's last annual report was issued a grant of £750 was announced towards a project to which the University Grants Committee and the National Institute for Research in Nuclear Science was also contributing.

It is important that Britain's organization for the support of research should be flexibly and also

effectively co-ordinated; the choice of project poses difficult problems also. With whatever justice it may be argued that our present expenditure on nuclear research, whether through the Atomic Energy Authority or more generally, is excessive, those who so argue are usually concerned at the neglect of a particular field. Dr. S. C. Curran, for example, was concerned at our neglect of space research and lack of a satellite programme. Here again we are concerned with a field calling for particularly costly equipment, but Sir Harrie Massey, in a recent statement referring to the growing interest in space research in British universities, suggested that such research could be considerably extended without distorting our scientific effort. This, he thought, was not a narrowly specialized field of work, but required more facilities, and collaboration in a European organization should not preclude a separate programme of research in Britain.

At the anniversary dinner of the Biochemical Society on March 29, Lord Hailsham said that it was important that not merely the structure but also the philosophy of our present arrangements for the organization and encouragement of research should be brought home to scientists. That is true enough, but it is no less important that the magnitude of Britain's effort should be seen to be in proportion to national needs, and it is from misgivings as to the scale of our effort that much of the present malaise arises. That is, of course, a main theme in the pamphlet, *Science and the Future of Britain*, issued in March by the Labour Party, but it is not simply a party political issue; it is fostered also by procrastinations and petty-mindedness that, as in such matters as scientific libraries, have already squandered resources in both finance and man-power.

Lord Hailsham quite rightly insists that the scientific planning of our research effort must be done by scientists themselves. Ultimately they must decide on the project and the means by which it is to be secured. That, however, ultimately turns on the availability of resources, and while, as Lord Hailsham said, the function of the Government is to see that the necessary research was proceeding and that its results were being applied, the fact that for the most part Government acts here indirectly through encouraging science in the universities, stimulating industrial research and organizing research institutes in fields where necessary, does not affect the fundamental issue of the availability of adequate resources.

The Minister for Science appears to be satisfied that the Research Councils, the Atomic Energy Authority, and the Advisory Council on Scientific Policy provide him with an adequate advisory structure, though some questions of ministerial relations could well be asked. He referred on this occasion particularly to the relations between the Department of Scientific and Industrial Research and the Medical Research Council and the University Grants Committee; the value of independent sources of finance for research is indisputable. Nevertheless, we come back once more to the question of the scale of the research effort in Britain. If the existing structure has proved its ability to prevent dissipation of our research effort on projects which are not easily justified in relation to viable national needs, it is the more important that its scale should be commensurate with real needs rather than political convenience. It is at the magnitude of our effort as well as at its deployment that Lord Hailsham should be looking, and making sure that departmentalism is no more an obstacle at ministerial level than in universities or elsewhere to the full exploration of new opportunities or a cover for waste and conservatism. Further, the implications of the relation between teaching and research also deserve examination, and if the President's Science Advisory Committee is right in its conclusion, some recent developments such as that at the Radar Research Establishment call for examination by the Minister for Science from that point of view if precious resources of man-power and equipment are to be used to the fullest advantage.

A REFERENCE BOOK FOR BIOCHEMISTS

Biochemists' Handbook
Edited by Dr. Cyril Long. Consultant Editors: Prof. Earl J. King and Prof. Warren M. Sperry. Pp. xxii+1192. (London: E. and F. N. Spon, Ltd., 1961.) 168*s*. net.

THIS handbook caters for fairly big-handed biochemists: it consists of almost twelve hundred pages of techniques, tables, facts, figures and references on a very wide range of biochemical topics, and includes the contributions of 171 authors.

The book is divided into six main sections. Section 1, called "Chemical Data", deals in the main with commonly used biochemical techniques like spectrophotometry, chromatography and manometry, but contains, in addition, information about isotopes, an excellent account of volumetric analysis (with many tables of buffer solutions and a very full list of acid dissociation constants), a brisk contribution on organic chemical nomenclature, and some useful data on the composition, structure and analysis of polysaccharides.

Section 2, "Data on Individual Enzymes", deals with nearly three hundred enzymes, which are arranged in groups according to the type of reaction they catalyse—hydrolysis, transfer, dehydrogenation, etc. Then follow a hundred pages on "Metabolic Pathways", giving a condensed and up-to-date account of important reaction schemes. This chapter is intended primarily for biochemistry teachers.

Section 4 (pp. 637–936) is called "Chemical Composition of Animal Tissues and Related Data". Presented mainly in the form of tables, this section takes us tissue by tissue through one, and sometimes several different species of animal to give an extraordinary wealth of data (46 pages are given to the composition of blood alone). Section 5 concerns itself with the chemical composition of plant tissues, and is considerably shorter than the previous chapter despite having "been given somewhat greater prominence than is customary". After a brief introduction on plant nomenclature and classification (a subtle 'dig' at biochemists who are all-'chemist' and no 'bio-' ?), subdivisions are made between one type of

chemical constituent and another. Individual presentations vary, but in general the compounds, with their chemical characteristics and natural sources, are listed with appropriate references, in tabular form. The book ends with a short section on "Physiological and Nutritional Data", which is rather on the fringe of biochemistry and has a fairly strong medical bias.

If, as the authors state in the preface, this book is meant to serve as a source of material for research biochemists, criticism could be levelled at some sections. The chapter on enzymes is so condensed and indigestible that probably both specialist and non-specialist alike will prefer to reach for 'Dixon and Webb'. One or two accounts of enzymes do not cover the subjects fully, and tend to be a bit unsatisfying because the selection of data has been too stringent. (The account of β, 2–1 fructanases, for example, does not even mention the work of Bacon and Edelman.) Also, in Section 1, perhaps it might have been a better idea in a book of this kind to have concentrated more on drawing up fuller lists of data (R_F values of more compounds in the chapter on paper chromatography, for example) rather than to have written so much on theory and technique, which are covered extensively in more specialized text-books. When the book concentrates on condensed information in the form of tables, figures and references (which is most of the time), it is excellent.

There are, however, one or two misprints; on pp. 953–4, '*Hordenum*' appears four times: it should be *Hordeum*. '*Liriodendro*' is printed instead of *Liriodendron* on p. 951. On p. 114 ribonucleose is written for ribonuclease, and on p. 515, the word 'succharides' might cause some hilarity.

The main strength of the book lies in its references, and in this respect alone it more than justifies the authors' claims that it should be regarded primarily as a source of biochemical material. If information is sought, one is almost certain to find it in the *Biochemists' Handbook*—and even if it is not there the references will indicate the source.

D. Recaldin

REVIEWS OF GENETICS

Principles of Genetics

By Prof. Eldon J. Gardner. Pp. vii + 366. (New York and London: John Wiley and Sons, Inc., 1960.) 60*s*.

Principles of Plant Breeding

By Prof. R. W. Allard. Pp. xi + 485. (New York and London: John Wiley and Sons, Inc., 1960.) 9 dollars; 72*s*.

Genetic Research

A Survey of Methods and Main Results. By Prof. Arne Müntzing. Pp. 345. (Stockholm: LTs Forlag, 1961.) n.p.

THESE three books are written for different readers. The first is intended "primarily for the college student taking his first course in genetics" and presents the fundamentals of genetics accurately in a straightforward and readable manner. The first eight chapters are concerned mainly with Mendel's experiments, the mechanics of the transmission of hereditary units, their interaction and the ways in which they can be located on the chromosomes by studies of linkage and recombination. Then, among other topics, the author deals with structural alterations of chromosomes, sex determination, mutation and alleles and compound loci. He indicates some present trends in chapters on physiological genetics and population genetics. One of the pleasing features of the book is the skilful way in which experimental results from both higher and lower organisms are woven into the text; and with equal facility, the student is given a hint of the usefulness of statistics and chemistry in modern genetics. It is a pity that the book is so expensive.

Prof. Allard's book is not greatly different in scope from some of the others on this subject. The reader is assumed to have a knowledge of cytogenetics, and emphasis is placed on principles of plant breeding rather than on breeding procedures for particular crops. After a brief discussion of patterns of evolution in cultivated plants and a general account of their reproductive systems, the author separates self-pollinated and cross-pollinated crops and, in each, in turn, deals with the genetic basis of breeding and with breeding methods. In my own opinion, Prof. Allard loses some possible clarity by not considering the roles of genotype and environment in continuous variation apart from mating systems. One of the most admirable sections of the book is that on breeding for resistance to disease, in which the interactions of host and parasite are very fully discussed; unfortunately, resistance to virus diseases is completely overlooked. The author writes in a vigorous and lucid style, but at times the subject-matter is condensed to the point of oversimplification; contrary to the author's statements, cultivated potatoes are, regrettably, classified into several species and many diploid edible bananas are sterile. Several Latin names are wrongly quoted and there are other printing errors that could well be corrected in subsequent printings.

Prof. Müntzing attempts in his book to cover the whole field of pure and applied genetics for the benefit of "everybody working with living material or teaching biology". Indeed, in slightly more than 300 pages, he deals with more topics than are included in the two other books under review. The reader will find chapters on all the traditional aspects of genetics —Mendelism and the developments therefrom, the structure of chromosomes, speciation, plant and animal breeding and eugenics. The facts are described accurately and clearly, but in order to cover so much ground many behaviours have to be treated in a somewhat cursory way; for example, the topic of self-sterility in plants is dismissed with a brief account of gametophytic incompatibility; a short description of the biochemical genetics of eye colours in *Drosophila* serves as a picture of the gene-controlled formation of pigments in plants and animals. Nevertheless, the account of genetical investigations in bacteria and viruses and the discussion of the nature of the gene are masterly condensations of available information. I was left with the impression that the book is too advanced for the amateur geneticist but, without a more extensive bibliography, falls short of being a satisfactory text-book for the intending professional. It is an unfortunate compromise. The book is well reproduced with excellent illustrations and diagrams. That the text is a translation is still evident; apart from clumsy sentences, words are sometimes wrongly used: "bacteriophage . . . creeps . . .", "electron microscopes . . . large power of dissolution".

Kenneth S. Dodds

PROGRESS IN CHEMISTRY

Modern Aspects of Inorganic Chemistry

By Prof. H. J. Emeléus and Dr. J. S. Anderson. Third edition, revised and reset. Pp. xi+611. (London: Routledge and Kegan Paul, Ltd., 1960.) 35*s*. net.

Radioactive Tracers in Chemistry and Industry

By Pascaline Daudel. Text augmented and revised by the author. Translated by Dr. Ulli Eisner. Pp. xi+210. (London: Charles Griffin and Co., Ltd., 1960.) 36*s*. net.

Annual Reports on the Progress of Chemistry for 1959

Vol. 61. Edited by Dr. R. S. Cahn. Pp. 476. (London: The Chemical Society, 1960.) 40*s*.

THE book by Emeléus and Anderson is now so well known that any detailed description of its contents is superfluous. The new edition has been carefully revised, new sections have been added, and some of the text in earlier editions which is now less important has been omitted, so that the size of the book has not been substantially increased. This is important for two reasons. First, the very reasonable price of a book of this character has been maintained. Secondly, the amount of material which can be dealt with in lecture courses and read by students is limited, and while a book of this size provides something which an average student may be expected to master, a larger book would be less acceptable.

Among the many new topics dealt with, mention may be made of a short but instructive account of ligand field theory, the chemistry of ferrocene and related compounds, sulphur nitride and its derivatives, and much new material in the chapters on co-ordination compounds. The last subject, with the main emphasis on chemistry, has always been a notable part of the book, and I know of no other account which can match it for clarity and completeness. In most of the other sections which were dealt with in previous editions, such as the boron and sulphur hydrides, significant new material has been added. The whole book is worthy of the highest praise from the point of view of the selection of material and also for its treatment.

One or two minor matters which were noticed are mentioned here not for criticism but in the hope that the authors may see their way to consider them in a future edition. I think the rather short section on molecular orbitals, which is quite largely drawn on in later parts of the book, will prove difficult to many students. The symbols for hybrid states on pages 61 and 69 are not clearly defined. If another four or so pages were added to this section it could be made clearer. The short table of dissociation energies on page 101 seems to be based on a quoted source of 1947, and it might have been pointed out that some of the values are still doubtful. I did not notice any reference to 5-valent manganese, and the section on metal hydrides on page 416 should be brought up to date by inclusion of the new preparation of cuprous hydride; the rather sceptical treatment seems now scarcely justified. The higher oxide of sulphur described on page 473 has been shown not to exist. In some cases the statement that "the structure is unknown" is misleading; there is little doubt, for example, that iodine dioxide is I_2O_4, since it is diamagnetic. The new formula for graphitic oxide does not seem to be mentioned. On page 52 bivalent americium is mentioned but on page 597 it is said not to exist. It now seems that N_2O_3 is $ON{\cdot}NO_2$ rather than a compound with an oxygen linkage as shown on page 466, and it is scarcely correct to say on page 468 of hyponitrous acid that "the structure has not been determined", since the dipole moment of its esters clearly indicates a trans-configuration. These are all small matters which little affect the accuracy of the book as a whole and the obvious care with which the information has been sifted.

The subject of radioactive tracers is now so important both in chemical laboratories and in industry that the short account of it by Daudel is welcome. The biological applications are not included. The text is clear and easy to read and outlines of laboratory methods are given which could enable a reader to judge whether a method is likely to be of interest in his field or not. This applies particularly to the industrial applications, since there is obviously a large field here which may not be very well known to those engaged in technical processes. The sections are followed by detailed references to the literature which seem to be brought up to the year 1955. The series of reports now being issued by the National Academy of Sciences, Washington, under the auspices of the National Research Council, will provide further information. It seems to me that this modest book should find a place in every works laboratory.

The *Annual Reports* issued by the Chemical Society continue to provide, in a commendably small volume, a survey of progress in chemistry which for compactness and completeness is unrivalled. All chemists have cause to be grateful to those who participate in the difficult task of surveying the fields of knowledge which they are so well fitted to traverse. The more recent policy of attempting to cover, however briefly, all the important work of the year, rather than to provide reviews of selected parts of it, which is possible in the case of inorganic and organic chemistry, seems on the whole to be a good one. In physical chemistry it is scarcely possible, and selected topics are treated from year to year, the material which has accumulated in the interval being brought together. The book is one which every chemist and every advanced student should have, and its relatively low price puts it well within his reach.

J. R. PARTINGTON

MEGALITHIC TOMBS OF FRANCE

The Prehistoric Chamber Tombs of France

A Geographical, Morphological and Chronological Survey. By Glyn Daniel. Pp. xx+282+32 plates. (London: Thames and Hudson, Ltd., 1960.) 70*s*. net.

TO win appreciation for some phase of antiquity on the strength of modern excavations is far easier than what has been undertaken here—where the antiquities concerned have been known for centuries, until only yesterday often excavated badly, and celebrated in a literature in which perverse and obsolete terminologies have run riot. Dr. Daniel, supported by his wife, and with backing from many quarters as well as friendly French co-operation, has for long been working towards a systematic account of the megalithic and related stone tombs of France, their form and contents, and their placing in the frame of European prehistory in the third and second millennia B.C., to which in general they belong. This book, by no means his first study of megalithic

structures, is his best so far. It is not too hard to read. Though he as well as others will certainly follow it with further work, yet he has broken the back of the subject's principal difficulties. These, above all in classification and terminology, were formidable in a manner made peculiarly acute by the history of this study among the French, which he traces in a sympathetic chapter. The core of the work then comes in the 150 pages describing the tombs themselves, using, from the multitude of some 6,000 recorded, the numbers intelligible to-day in each of seven main regions : the North, Brittany, the Centre-West, the South-West, the South, the Centre, and the East. To visit these or their remains, perhaps 1,500 of them, the Daniels have travelled, tramped, recorded and tramped again, cheerfully making friends by friendliness and amassing information; the mass is here given to us critically sifted and classified, and steadfast readers will find that it makes sense. The steadfastness is of course put to some strains, though the worst is not the terminology, because Dr. Daniel has worked so hard to straighten the tangle of both French and English theoretical and descriptive terms. The most unnecessary is his assumption that readers can do without any sketch of the background in Mediterranean and Iberian lands whence French tomb-building was initially inspired. For of his two main categories, passage-graves come all from Iberia, whether Atlantic-wise to Brittany or Mediterranean-wise to the South, while gallery-graves may now be Iberian too, now adapted in the South from rock-tombs in Sardinia. One is supposed to have looked up classes of pottery, too, such as Bell-beaker or Polada, elsewhere beforehand.

The same applies to the chronology. For nearly all the dates used are borrowed, without reason for them given, from other lands. In the second millennium, most are slightly too low; in the third, the whole scheme may need drastic raising to meet carbon-14 determinations, which began to invade the megalithic field just when the book was finished. Yet this again means that it is timely: it tells us *what* the pattern is that carbon-14 may alter; and without that, the new method would have looked like making confusion worse confounded. The book deserves a serious welcome.

C. F. C. Hawkes

MATHEMATICAL DELIGHT

The Language of Mathematics

By Dr. Frank Land. Pp. vii + 264. (London: John Murray (Publishers), Ltd., 1960.) 21*s*. net.

In an essay which formed part of the symposium "The Quality of Education", Dr. J. Bronowski put forward in 1947 the thesis that mathematics was a language. For many people this statement would seem to be an exaggeration if their remembrance of the subject was little more than a series of tricks designed to get the 'right answer' by the application of routine processes. Yet a little reflexion will show clearly that some, at least, of the essential constituents of a language are certainly present. There is a vocabulary (which like most may be merely remembered); there is certainly a grammar, the rules of which must be rigidly followed. It is a necessary means of communication about the real world, and here the limits need not be set within national boundaries. What has been so long neglected is the æsthetic appeal of this discipline, rigid in its logic but thrilling in the power of its 'literature' and its 'poetry'. The past thirty years has been a time of amazing development at the frontiers of knowledge in this field, but there has also been astonishing progress in the methods of teaching at all stages from the primary school onwards.

Dr. F. W. Land, the professor-elect in education at the University of Hull, carried out an investigation into the "attitude and attainments of 2,000 grammar school leavers", and the study revealed the need to present the fundamental principles of mathematics in a new light for the mature reader. The Shell International Petroleum Co. made it possible for Dr. Land to embark on this project, and the present volume is his very successful attempt to satisfy this need. The book is published in two bindings, and there is now available a set of exercises which will add to the profit of those who approach it with serious intentions.

It is in every respect a delightful book; merely to turn its pages is an attractive occupation and the reader is drawn again and again to browse, to ponder and then to read with understanding. Little in the way of prior knowledge or acquired ability in mathematics is necessary from those who begin. If they are prepared to think as they read, the author's enthusiasm and clarity of expression, supported by Margaret Clark's beautiful illustrations, will carry them along. I read it at a single sitting and have no hesitation in saying that it should form part of the general studies course in every sixth form, as well as being a source of pleasure and profit to every intelligent adult.

The first six chapters are devoted to arithmetic, but they range far outside the limits of commercial calculations, which is all that so many students know about the concept of number. There is an attractive treatment of the problems of the calendar, and a careful discussion of the British system of units. Then come four chapters concerned with the generalizations which we know as algebra, though here again the range is wide and the treatment exciting; the use of graphs leads to such unexpected considerations as the whirls on the shell of *Nautilus* and the florets in the heads of Compositae. The second fundamental concept of mathematical thought, the concept of space, includes within its two chapters much that is traditional in geometrical teaching, but map projections and the four-colour problem, some elementary topology and the applications of Euclid's golden section, show how Nature has her laws which man may understand and use though he cannot control them. The final chapter, on statistics, is perhaps the only part of the book in which considerable demands are made on the reader, for though the treatment of normal distribution is easy and interesting, the development of correlation and the use of scatter diagrams may cause some readers to put aside the last few pages for 'another day'.

A tribute is due to Will Carter, who designed the volume; his collaboration with the printers has produced a book of lasting value and immediate appeal. The proof reading was carefully done for there are few misprints. Such blemishes as the statement (p. 171) that the three angles of a triangle "add up to a straight line", and the drawings (p. 156), which are neither isometric nor perspective, can easily be removed with a second edition, and this should soon be needed. The book ought to be in every public library in the country—or rather its place on the shelves should permanently be empty.

W. J. Langford

FUTURE OF NUCLEAR PHYSICS IN AUSTRALIA*

By Prof. E. W. TITTERTON, C.M.G.

Research School of Physical Sciences, Australian National University, Canberra

RESEARCH into nuclear physics nowadays falls into two main categories—what I shall call low-energy and high-energy nuclear physics. In general terms I would define low-energy nuclear physics as embracing the energy-range for bombarding particles between zero and some 50 MeV. Under these conditions the incoming bombarding particle is of sufficiently long wave-length to see the target nucleus as a whole; that is, we are able to study the properties of all nuclei available to us. At higher energies, up to the maximum presently available from accelerating machines, which is 25,000 MeV. or 25 GeV., the wave-length of the incoming projectile is small compared with the nuclear size and the interaction is no longer with the nucleus as a whole. To the bombarding particle, all nuclei look the same—just an assembly of neutrons and protons. The interactions are nucleon–nucleon interactions, and tell us little about nuclear structure; among other things, they lead to the production of mesons and strange particles which are associated with nuclear force fields and which, therefore, are of great importance.

To see what the Australian role in these fields is likely to be in the future, it is perhaps easiest to survey, first of all, the present position. Early in March this year, a major conference on nuclear physics was held in the Australian National University, Canberra. In many ways it marked a turning-point for Australia—partly because it was held to coincide with the final stages of erection of a new accelerating machine at the Australian National University, and partly because, for the first time, outstanding overseas research workers felt it to be worth while to travel to Canberra especially to take part in the meetings and learn what was going on in the field in Australia. In the four days of the meeting, virtually all current work in Australia was reviewed.

Sixty-two papers were read, of which twenty-eight came from the Australian National University, eleven from the University of Melbourne, six from the University of Sydney, four from the University of Adelaide and one each from the Universities of Tasmania and Western Australia; the remainder were papers given by overseas visitors. The vigorous growth of the work in the country can be judged from the fact that at a similar conference organized by the Australian National University in 1958, the total number of papers presented from all sources was twenty-eight; the output of work increased considerably in the three-year interval.

In Table 1 a summary is given of work going on in the Australian universities. It will be seen that of the ten universities, seven have some work in the field, if one includes work in cosmic-ray physics. In recent years, as is shown, there has been a very strong development of theoretical nuclear physics in the country. This came about partly because of the intrinsic importance of such work, but more importantly because big capital investment is not required and, apart from computer time, expenditure for theoretical work is limited to salaries and the provision of office accommodation. The situation is quite different in the case of experimental nuclear physics, especially if one leaves aside the rather specialized work in cosmic rays. Worth-while work in nuclear physics necessitates big capital expenditure for laboratories, accelerating machines, detecting equipment and data processing, while considerable staffs of technical assistants are required to maintain the very complicated equipment and aid the experimental work. Because of this heavy demand on money and on staff, such work is pursued only in the University of Melbourne and at the Australian National University. The former has four accelerating machines—one variable-energy cyclotron capable of yielding protons up to a maximum of 12 MeV., two electrostatic generators capable of voltages up to 800 KeV., and an electron synchrotron yielding a peak energy of 18 MeV. Of these, the cyclotron is a major experimental tool, and has, for some time, been turning out important experimental results.

Table 1. Summary of Nuclear Research Work Now in Progress in Australian Universities

University	Scientist in charge	Type of work
Australian National University	Sir Mark Oliphant	Particle (high-energy) physics including the design and construction of a 10,000-MeV. proton accelerator.
	Prof. K. J. LeCouteur	Theoretical work in nuclear physics.
	Prof. E. W. Titterton	Experimental work in nuclear physics.
University of Melbourne	Prof. C. B. O. Mohr	Theoretical work in nuclear physics.
	Prof. D. Caro	Experimental work nuclear physics.
	Dr. V. D. Hopper	Experimental work in cosmic-ray physics.
University of Sydney	Prof. H. Messel	Theoretical work in cosmic rays.
	Prof. S. T. Butler	Theoretical work in nuclear and particle physics
	Prof. C. B. McCusker	Experimental work in cosmic-ray physics.
University of Adelaide	Prof. H. S. Green	Theoretical work in nuclear physics and high-energy physics
University of New South Wales	Prof. J. M. Blatt	Theoretical work in nuclear physics.
University of Tasmania	Dr. A. G. Fenton	Experimental work in cosmic-ray physics
University of Western Australia	Dr. H. Thies	Certain photonuclear experiments.

* Substance of the presidential address delivered to Section A of the Australian and New Zealand Association for the Advancement of Science meeting in Brisbane, May 30, 1961.

At the National University there are four accelerating machines, including a 1,200-KeV. Cockcroft–Walton accelerator, an 8-MeV. cyclotron, a 33-MeV. electron synchrotron and a 12-MeV. tandem electrostatic accelerator completed in May of this year. Nearing completion is a 2-MeV. Van de Graaff generator which will serve in the dual role of an accelerator in its own right and also as an injector to allow helium ions to be accelerated in the tandem. Under construction there is a 10-GeV. (10,000 MeV.) proton synchrotron which will be powered by a giant homopolar generator capable of currents in excess of a million amperes.

This last machine, which is being constructed by a group working under Sir Mark Oliphant, incorporates novel features, many of which were essential to allow the machine to be built with the funds and staff available. Thus an orbit of about 30 ft. diameter was chosen, and this requires restraining magnetic fields at the orbit of 80,000 gauss or so. Such fields cannot be achieved with an iron magnet, and an air-cored system was therefore chosen. This necessitates currents of peak value near to 1½ million amp., requiring a homopolar generator with liquid metal brushes as power source. The penalty which one pays for this 'poor man's' high-energy accelerating machine is a low pulse-rate—approximately 1 pulse/10 min. In competition with overseas machines in the same energy-range, where pulse-rates are one every 1 or 2 sec., this means that there is a severe intensity disadvantage. The field of experimental work in high-energy physics with this machine when it is completed will therefore be limited.

The homopolar generator, however, will be a most useful power source for other purposes—for example, certain investigations in the plasma physics field.

This accelerator of Oliphant's is likely to be the only one built in the high-energy field in Australia. The reason is that investments now being made for high-energy accelerators of 10 GeV. and upwards overseas are in the range of £A.10 million–£A.100 million with similar sums of money per installation required for ancillary experimental equipment. Moreover, the teams of people required to build, operate and use such equipment approach, for one installation, the entire physics staffs of the combined Australian universities. It is therefore clear that in the future, Australia will be unable to compete with the big nations in the field of high-energy experimental particle physics. Some work can go on profitably in cosmic-ray physics, but this is likely to be on the more geophysical aspects of the subject rather than in the nucleon–nucleon interaction field where, again, the intensity advantage and flexibility of experimental arrangement make an accelerating machine the highly preferable tool. There is no reason, however, why effective theoretical work in the high-energy field should not be carried out in Australia, and it is encouraging to note that small groups are already in being in Adelaide, Canberra and Sydney.

To return to the field of low-energy nuclear physics, I should remark that, in addition to the work going on in the universities, there is a group at the Australian Atomic Energy Establishment, Lucas Heights, Sydney, whose task it is to make certain nuclear measurements of importance to the atomic energy programme. It is called the Reactor Physics Division, and was responsible for the nuclear measurements made in Australia's first reactor *Hifar*, and it has now acquired a smaller reactor named *Moata* for the needs of its own work. Most of its work is of an applied nature, but some of its studies in neutron physics, for example, neutron diffraction, will be of wider interest.

A joint Universities–Atomic Energy Commission organization called the Institute for Nuclear Science and Engineering is now beginning to operate effectively. It has offices and laboratories at Lucas Heights, and members of staff of any Australian university desiring to use the unique facilities at Lucas Heights are encouraged to go and work there. Although the Institute will be able to support some nuclear physics work, this is likely to be a small part of its overall programme since the nuclear problems which can be tackled using a fission spectrum in a reactor are limited in variety. It is to be expected that most experimental effort will go into chemistry, metallurgy, solid-state physics, biology and medicine, and perhaps geophysics.

It will be apparent, then, from this brief survey that the future of nuclear physics in Australia will reside, on the experimental side, in the fields of low-energy nuclear physics with some peripheral activities in cosmic rays and plasma or thermonuclear physics. But there is no reason why, on the theoretical side, work should not range over the whole field providing always that our theoretical colleagues give adequate backing to the low-energy experimental programmes going on in the country.

It was for reasons of the type outlined above that in considering the future programme at the Australian National University we chose to make a major investment in the field of low-energy nuclear physics by building a tandem electrostatic generator which is the most flexible and sophisticated tool available to the experimenter at the present time.

But, first, I want to offer reasons why I believe it is essential to have at least two flourishing schools of experimental nuclear physics research in the country.

The training received by a graduate in going to a Ph.D. degree in nuclear physics is about the broadest I know. The student must learn to handle a wide variety of equipment—electronics, both valve and transistor, vacuum physics, thin film preparation, accelerating machines, a wide variety of light and ionization detectors, multichannel memory storage devices, data processing systems and so on—merely as tools for doing the job of an experiment in nuclear physics. Then he must have an adequate understanding of the basic theory to be able to interpret the results properly. I am inclined to agree with Dr Louis Rosen, a recent visitor to our Laboratory from the U.S. Atomic Energy Commission, who said: "We find that training in nuclear physics is the best a physicist can get. Such men have great breadth of outlook and a remarkable diversity of knowledge. They can turn their hands at any time to solve difficult problems of technology which continually arise in the country".

This point was demonstrated most effectively during the War, when it was found that the nuclear physicists recruited from the universities, both in the United Kingdom and the United States, rapidly took over the leadership in the development of radar and operational research methods before going on to develop the nuclear reactor—the power source of the future—and the uranium isotope separation methods required to obtain the highly fissile materials plutonium and uranium-235, necessary to manufacture the nuclear bombs which finished the War.

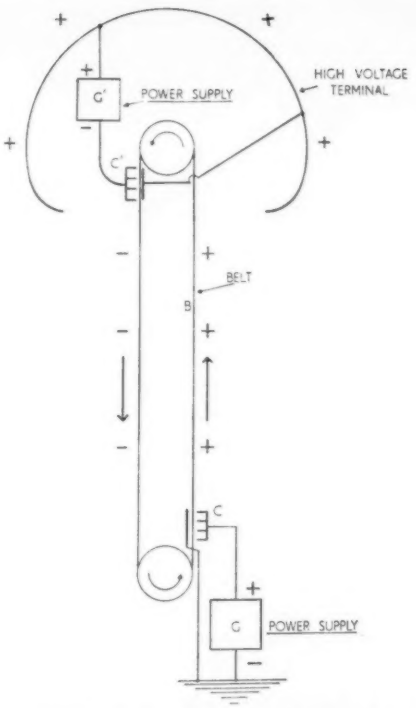

Fig. 1. Schematic diagram of the original (single ended) Van de Graaff accelerator. Charge from a power supply G is sprayed into a moving belt B and carried to a hollow spherical terminal T where it is removed. Charge of the opposite sign can be sprayed into the belt from the power supply G' and carried from the terminal if desired. The practical voltage limit to such a machine is 6–7 million volts

Out of these nuclear developments has grown the biggest industry in the world—the nuclear or, as it is popularly called, the atomic energy industry. This will provide an ever-increasing proportion of the world's power resources as fossil fuels become depleted, and it will become essential in the long-term development of Australia. The most promising energy source after fission resources become exhausted—and they will have a life-time of some 50,000 years—is again a nuclear one. It arises through the synthesis of heavier elements from lighter ones through fusion or thermonuclear reactions similar to those we now know provide the major source of stellar energy.

Fission and fusion then are two compelling practical reasons why any forward-looking country must study low-energy nuclear physics.

My final reason is probably the most important. Although we have an ever-increasing knowledge of the properties of nuclei, we are still far from understanding the detailed structure of nuclei, and the seat of the very strong nuclear forces which bind the nucleons together in a nucleus. It is a major aim of nuclear physics research to try to provide such understanding.

To illustrate the scale on which one needs to operate in low-energy nuclear physics these days, I can do no better than to describe our new installation in Canberra. The ingredients for nuclear experimentation are: (1) appropriately prepared targets; (2) an accelerating machine with which to produce the fast particles with which to bombard the target; (3) detecting and recording equipment to observe the particles or radiation leaving the target as a result of nuclear reactions.

Target preparation is an art in itself, and will not be discussed here, but I should say something about the other two since this is where the big expenditure and effort lie.

To-day, for precision work in nuclear physics, the choice of the experimentalist is for an electrostatic accelerator of the type first built by R. J. Van de Graaff in the United States. Such machines are exceptionally useful for investigating properties of nuclei because they produce highly collimated beams of bombarding particles the energy of which can be varied continuously and smoothly, and they yield particles which are nearly monochromatic in energy.

The operating principle of a conventional electrostatic generator is illustrated in Fig. 1. An endless belt, B, of rubberized fabric, runs over two pulleys placed one above the other. Electric charge from a power supply, G, is sprayed on to the lower end of the rising half of the belt by means of corona discharge from points on a metal comb, C. The charge is carried up by the belt and at the top enters a spherical hollow metal terminal, T, to which the charge is transferred from the belt by means of another comb, C'. Alternatively, charge of the opposite sign can be sprayed on to the belt from another power supply, G', inside the terminal. In this way, the positive charge on the terminal is continually built up, the potential obtained being limited only by the rate at which it leaks off, or by electrical breakdown to the surroundings.

An evacuated acceleration tube, consisting of alternate sections of glass and metal, connects between the terminal and earth potential. An ion source mounted at the upper end of this tube supplies positively charged particles such as protons, deuterons or tritons, and these are accelerated through the potential difference between the terminal and the ground. The higher the maximum voltage to which the terminal is required to be raised, the longer the accelerator tube needs to be and the larger the diameter (and therefore the electrical capacity) of the terminal must be to withstand the voltage stress.

In actual fact it turns out that 6–7 million volts is the practical limit, partly because, beyond this figure, the total stored energy in the terminal ($\frac{1}{2}$ CV^2) is so great that any odd arc discharge from it to the surroundings causes great damage to the system. Unfortunately, 6 million volts are insufficient for many of the problems we want to attack, and it became necessary to improve on this. The machine just completed at the Australian National University will allow particles to be accelerated to more than 12 MeV. and is a tandem accelerator. It employs ion exchange techniques for increasing beam energy which were suggested many years ago by W. H. Bennett, but which at the time were not practical. Renewed interest in the idea occurred when physicists learned how to produce negatively charged hydrogen ions, that is, protons to which two electrons have been attached. Such ions can be produced with an intensity of about 2 parts in 100 when a beam of protons, accelerated through a potential difference of about 20 kV., is allowed to pass through hydrogen gas. Under such conditions the velocity of the protons corresponds approximately to the velocities of the outer orbital electrons in the gas atoms and leads to the formation of the negative ions. This negative ion beam is then analysed magnetically so that unwanted

particles, such as neutral particles and electrons, are removed from the ion beam. The clean ion beam is then injected into the tandem accelerator, as indicated in Fig. 2. The negative ions are accelerated by the positive voltage on the central high-voltage electrode of the machine. When they reach this terminal the negative ions are moving with a centre of mass velocity which is large compared with that of the outer electrons about a nucleus. In these circumstances, by allowing the ion beam to pass through a tube containing gas (called the 'stripper' tube), electrons are stripped off and the emergent beam consists of positively charged particles. The gas pressure in the stripping canal can be maintained at a low enough value so that the energy lost in passing through the gas is negligible (less than 0·5 keV.). The positive ions so formed are then pushed away from the positively charged central terminal and are accelerated in the second half of the stack. The energy of the emergent beam is therefore twice that of the terminal voltage and, with 6 MV. on the terminal, the emergent beam has an energy of 12 MeV.

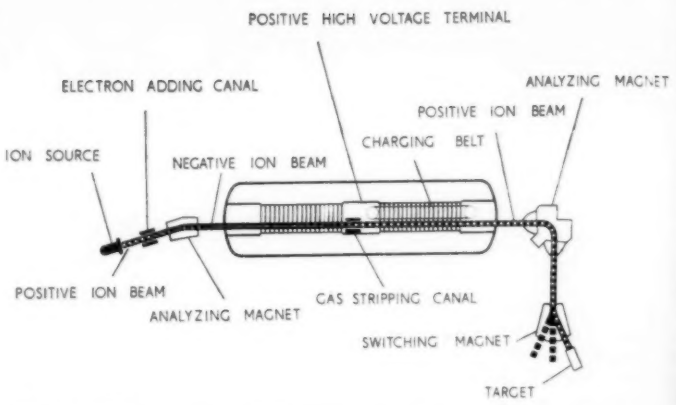

Fig. 2. Functional diagram of the operating principles of a tandem electrostatic accelerator

The tandem generator has many possibilities for experimental work. It is the most flexible accelerating machine yet built, for the reason that the ion source and the target assembly are both at earth potential and readily accessible to the experimenter (the ion source in the conventional Van de Graaff generator, Fig. 1, is in the high-voltage terminal within the pressure shell of the machine and is inaccessible).

Proton and deuteron beams with energies of about 12 million volts and oxygen ions with energies of 30–40 MeV. can be readily obtained. In the more distant future, ion beams of lithium, fluorine, and any other element capable of forming negative ions (a criterion which includes about half the Periodic Table) may be produced.

Unfortunately two very important projectiles, helium-4 and helium-3, do not form negative ions long-lived enough to be accelerated with intensity comparable with protons, deuterons or tritons. In the near future, therefore, we are planning to install an injector which will make such acceleration possible. This will use a conventional 2 million volt electrostatic machine in which He^+ ions are accelerated to about 700 keV. (which is an optimum figure for the purpose), neutralized in a charge-exchange canal, injected into the tandem machine and allowed to coast, without gaining energy, to the high-voltage terminal. There, as before, they are stripped of two electrons, becoming He^{++}, and in falling through the potential difference of 6 MV. in the second accelerator tube gain 12 MeV. of energy to emerge with a total of 12·75 MeV. The injection machine will be mounted on a turn-table and railway arranged so that, when not in use for helium-ion injection to the tandem, it can be run back into an adjacent laboratory, rotated through 140° and used as a 2-MeV. accelerator in its own right in conjunction with an appropriate analysing magnet which can switch the beam into either of two directions for experimental work.

This example of the helium-ion injector illustrates one of the great advantages of the tandem accelerator. Because the ion source is at ground potential and readily accessible, there are virtually no limits to the power consumption, size and complexity of the injector system. For example, μsec. time of flight techniques (which are important in many experiments) can be applied very readily with the tandem generator, since radio-frequency deflexion and bunching can be carried out on the low-energy beam before injection. Further, it may be possible in the future to polarize the nuclei of negative ions and eventually produce beams of polarized ions, which would open up entirely new fields of experimentation.

Preliminary experiments with the tandem accelerator show that the peak-to-peak ripple at 5·1 MV. on the high-voltage terminal under beam conditions is 1·6 kV. with a slow drift of 0·5 kV. The voltage stability of the beam is therefore better than one part in a thousand, and to make use of this it is necessary that precision detecting equipment be available in the target area.

Two major units will be available for precision experiments—a 24-in. double focusing 180° spectrometer and a 65-cm. Buechner particle spectrograph, both of which can analyse the charged particles leaving a target under bombardment. The former incorporates a 20-ton magnet having a specially shaped magnetic field of value quadratically dependent on the radius of the particle trajectory in it. In cylindrical polar co-ordinates there arises a radial component of the field in addition to the z-component, enabling focusing in the z-direction to be effected as well as radial focusing. By shaping the field so that the coefficient, α, of the first-order term of $H_z(r)$ is exactly one half, the radial and z-focuses coincide for all values of object distance, and in our spectrometer object distances 9–26 in. from the field boundary can be chosen. The size of the image given by such a magnet depends on the object size and aberrations. Aberrations can be reduced considerably by choosing the coefficient, β, of the quadratic term in $H_z(r)$ correctly. The pole-face profile is therefore complicated, but successful achievement leads to a greater solid angle of particle acceptance. In the present instrument, for equal object and image distances the solid angle of acceptance is 0·01 steradian at 0·1 per cent energy resolution for fields up to

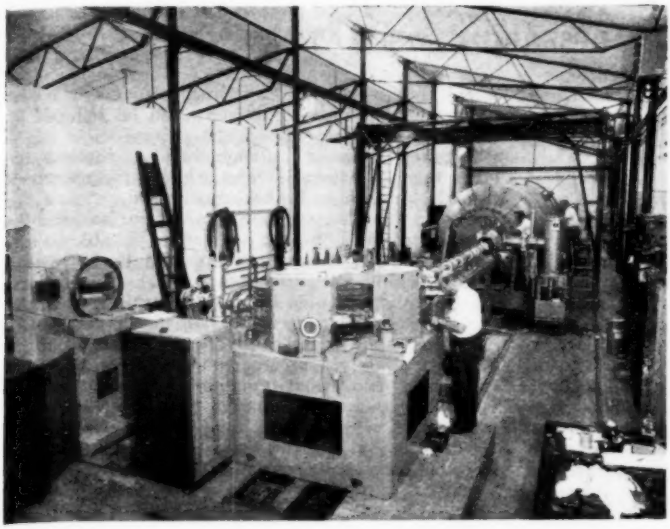

Fig. 3. The tandem accelerator at the Australian National University viewed from the high-energy end. In the foreground is the 90° analysing magnet. On the left (not yet connected to the machine) is the switching magnet which allows three beams, + 25°, 0 and − 25°, to be obtained. The accelerator pressure vessel is in the background

10,000 gauss—sufficient to analyse protons of energy up to some 25 MeV. In use it is mounted on a trolley and is fed with current through flexible cables so that it can be rotated around a target from + 10 to − 155° to analyse reaction products as a function of angle.

The second major piece of equipment—the Buechner spectrograph—will be installed early in 1962. It differs from the spectrometer in that it will accept and analyse particles having a wide range of energies simultaneously; in fact, at one field setting particles in the energy-range from E to 2·4 E are recorded. In this case the magnet employs a uniform field having a circular boundary which focuses particles from the target located outside the field along a hyperbolic focal surface also outside the field after a mean deflexion of 90°. Again an energy resolution better than 1 part in 1,000 can be achieved and recording is by nuclear emulsion, the plate being bent to take up the shape of the focal surface. With this device the solid angle of acceptance is much smaller—about $1{\cdot}4 \times 10^{-3}$ steradian—but the photographic emulsion detector can integrate the particle spectrum over a lengthy run and over the wide range of energies. At a field of 12,000 gauss the spectrograph will record protons and α-particles up to 35 MeV. and deuterons up to 17-MeV. energy. The magnet weighs 16 tons and can be rotated about the incident beam from −10° to +130° to enable angular distributions to be measured.

In addition to the spectrometer and spectrographic methods of analysing reaction products, the more conventional electronic—gas counter, scintillation counter—and nuclear emulsion techniques will be employed.

The more recently developed silicon surface barrier detectors are likely to have very wide application; they are compact, with a 100 per cent detection efficiency for charged particles, a linear energy-dependence over a wide range and extremely short pulse rise time. In some of those we have made in the laboratory, energy resolutions of 1 per cent have been achieved for α-particles and this necessitates the use of low-noise amplifiers. Unfortunately, at present, the layers are thin for protons and detect linearly only up to some 3 or 4 MeV. These counters, however, are ideal for many purposes—particularly the simultaneous study of particles emitted at a number of angles from a target.

Most detectors present a problem of pulse-height analysis—determination of the spectrum of pulses arising from them and multichannel kicksorters capable of accepting the data, sorting it into 400 or 512 channels, storing it for the duration of a counting period and then printing it at the end of the run are necessary. For reliability these devices are largely transistor operated; we have two in operation and will install another two in the course of the next year.

The foregoing review will indicate that in the immediate future experimental nuclear physics is well cared for in the Melbourne and Canberra laboratories, while theoretical nuclear physics is flourishing both in these laboratories and in other universities too (Table 1). The question may be asked whether there should be more work in the general field. This must be answered in relation to the problem of a balanced development of physics in the country. To set up a new and effective Department of Experimental Nuclear Physics would require a capital investment of between half a million and one million pounds, with running expenses of perhaps £150,000 per annum. However enthusiastic one may be about the discipline, it must be admitted that in the present stage of national development, such funds put into other, possibly less-glamorous and less-spectacular but no less-important, parts of the physical sciences, would ensure a better balance in the overall development of physics in Australia. But we, like other Western countries, will have to build further universities in the next two decades and it may be that expansion of nuclear activities could take place then.

Along with some others of my colleagues, I have become concerned at the way research in physics is being represented to the public. In the public mind there is a tendency to believe that the building of nuclear reactors, the placing of satellites into orbit, the production of nuclear bombs and the plans for men to visit the Moon are science. Such projects do, of course, use the knowledge gained in past research, but at best they are applied science, and more generally steps in a development programme; as such, of themselves, they add little to our store of fundamental knowledge.

Pure research in physics cannot be carried out in the full glare of publicity of Press, radio and television. There is nothing certain about it; it flourishes quietly and methodically in the laboratory. Its results go to acknowledged responsible scientific journals both in Australia and overseas, and its merit is judged by colleagues in the international community of scientists.

THE ROAD RESEARCH LABORATORY

OPEN days were held by the Road Research Laboratory (Department of Scientific and Industrial Research) on Wednesday and Thursday, May 3–4, 1961.

The work of the Traffic and Safety Division was shown at the new Road Research Track at Crowthorne, Berkshire. The track is three miles long and is in the form of a figure eight with a large paved area 900 ft. in diameter in the centre. A detailed description of the track has been given in *Nature* (188, 894; 1960).

The display at Crowthorne comprised static exhibits in marquees and in an underground laboratory, and several demonstrations of the work on vehicles, skidding, road user behaviour, and traffic.

In one marquee, equipment used in traffic studies was on view and included traffic counters, a recording speedmeter, data recorders and multi-channel telemetry equipment for traffic studies over wide areas.

Traffic and Safety

Demonstrations were given of some of the work undertaken in the application of electronic guidance to motor-vehicles.

A car was driven along a bend on part of the track in which detector loops were buried in the road. The passage of the car caused lights to be switched on, and remain on until the car was three loops away. In fog such a device could reveal to a driver in a car how far ahead the road was clear. Similar detectors could be used to check the speed of passing vehicles and warn drivers when they were exceeding the speed limit.

In another demonstration of the use of electronic control, a car with its windscreen blacked out was driven along a straight section of the track. The driver was guided by his instruments, which were actuated by two coils at the front of the vehicle to pick up impulses from a guide wire on the ground. An audible signal was transmitted to the car, and for the purpose of demonstration was amplified over a loudspeaker system. When the driver was on course the signal was heard loud and clear, when the driver moved away from his predetermined course the signal was weak. The transmission is actually made from the roadside via the guide wire beneath the centre of the track. On a road equipped with a guide wire this system could be used by traffic police to communicate with a motorist who had a suitably modified radio installed in his car.

A possible development from these investigations, which are at present in their early stages, would be to link the signals direct to the controls of the car and thus drive the car automatically. In fact, in some runs the car steered itself entirely by electronics and was kept within 6 in. of the guiding cable.

The greater resistance to skidding, and the shorter braking distances, that can be achieved with tyres having tread rubber with a high hysteresis loss was demonstrated by two identical cars, one following the other and about 30 ft. apart, at a speed of about 40 miles per hour on a wet, slippery section of the track. At a given signal, each driver applied his brakes as in an emergency stop; the car equipped with conventional tyres travelled farther in coming to rest than the one fitted with tyres with tread rubber with a high hysteresis loss. Experiments have shown that by this means braking distance can be reduced by about 14 per cent.

Another series of demonstrations showed the advantage of fitting devices which prevent wheels from locking when brakes are applied. A car was driven at 45 miles per hour on a wet surface, and the driver applied his brakes as hard as possible to lock the wheels. When using Maxaret units to prevent wheel locking the driver did not lose directional stability and was able to retain steering control and bring the car to a stop in a much shorter distance.

To help analyse the cause of accidents the Laboratory is using a camera suspended on a 40-ft. collapsible pole which protrudes from the roof of a van. The camera photographs, from above, the exact position of vehicles that have been involved in an accident. By this means it is hoped to obtain information on deficiencies in vehicle design and road layout.

The Laboratory has been co-operating with the glass industry in attempts to improve vision through toughened glass windscreens when they shatter. By reducing the fragmentation of the glass to between five and ten particles per inch in the part of the windscreen immediately in front of the driver he is given reasonable vision when the glass is shattered. The effectiveness of the new glass was demonstrated by driving a car with a shattered windscreen and manœuvring it around obstacles at about 20 miles per hour.

The problem of getting information across to road users in the most efficient way was shown by a demonstration in the central area. Signs were mounted on a car which was driven towards the spectators, who were then invited to record by pressing push-buttons when they could read the signs; the distances at which they could do this were clearly indicated. Signs with different types of lettering were used.

About a hundred people are killed each year on the roads in Britain as a result of cars striking lighting columns. The Laboratory is examining the design and positioning of columns with the view of minimizing injuries to road users. A film showed the effect of an 8 h.p. car hitting four types of lighting column—concrete, tubular and thin sheet steel, and aluminium.

The vehicle detectors now in use on the road can register moving vehicles only; they cannot therefore distinguish between dense stationary traffic and the complete absence of vehicles. Two devices that can detect the presence of moving and stationary vehicles are now under development; both depend on the change in the magnetic field produced by a nearby metal object.

The Laboratory has been examining the possible applications of vehicle-presence detectors and a demonstration was given of the detection of congestion in a roundabout. The equipment was also used to indicate imminent 'locking up' at a signal-controlled roundabout.

A prototype device was shown that is being used in experiments with vehicle-actuated traffic signs on high-speed roads. As shown in a series of demonstrations, it ensures that the traffic signals will not change from green to amber when a driver approaches an intersection at such a speed that he can neither

stop safely nor clear the intersection before the 'red' appears. A transistorized form of the device is now under development by manufacturers.

Materials and Methods of Construction

The work of the Materials and Construction Division was shown at Harmondsworth, Middlesex, where research is undertaken on pavement design, soil moisture and surface drainage, bituminous materials, concrete and special problems such as those arising from snow and ice and the measurement of surface irregularity. The Laboratory's Tropical Section is also at Harmondsworth.

A number of exhibits dealt with the problem of designing road pavements in relation to foundation conditions and the traffic to be carried. The Laboratory has many full-scale road experiments in progress in which the performance of short sections laid on heavily trafficked routes is being studied. The main variables are the thickness and type of both surfacing and base in the case of flexible roads and the thickness of slab and weight of reinforcement for concrete roads. Results from experiments which have been in progress for periods up to twelve years were shown.

Severe frost, which occurs on average every 10 years, can cause damage to road foundations. In a refrigerated road full-scale freezing tests on a typical road section were in progress. Apparatus was also on view for studying the moisture retention and swelling of soils, and photographs showed the relation of this work to practical problems associated with pavement failures.

The function of a road pavement is to spread the wheel-loads so that the stresses in the soil and other layers of the road structure do not cause unacceptable permanent deformation. This load-spreading depends on the elastic properties and thickness of layers in the road. The stresses in the structure and in the soil can be determined theoretically and they are also being measured by equipment developed at the Laboratory. Exhibits were concerned with dynamic stresses measured under different forms of flexible construction and with the effect of repeated applications of these stresses.

In one building were assembled rainfall recorders and flow-measuring apparatus devised at the Laboratory for use in research on surface-water drainage. The main objective of this work is the design of drainage systems both in relation to their efficiency and their cost. Apparatus is being devised for printing hydrological data directly on to tape for analysis by an electronic digital computer. A working model was shown of a novel automatic recording salt-velocity apparatus devised jointly by the Road Research Laboratory and the Hydraulics Research Station. The apparatus comprises a device for injecting charges of salt solution into the sewage (each charge is triggered at a pre-set depth of flow by a water-level recorder), two pairs of electrodes in the sewer and apparatus for recording the change in conductivity as the salt charges pass the electrode. This enables the velocity of flow between the electrodes to be measured for a range of depths; a relation between the rate of flow and the depth of flow is then deduced. Also on display was an analysis of records of rainfall and run-off for twelve catchment areas, employing the rational (Lloyd-Davies) method, the unit-hydrograph method and the Road Research Laboratory hydrograph method. Two methods of calculating sewer sizes for design purposes were on show; both methods were devised at the Laboratory from the results of research into rainfall and run-off. In one method use is made of an electronic computer.

Bituminous binders are viscous liquids adhering to the surface of roadstone. Viscosity and adhesion are therefore two primary properties, and an exhibit described problems connected with the measurement of viscosity, the effects of water on adhesion, and the use, in wet weather, of adhesion agents to prevent damage to a surfacing. The resistance of the binder to hardening under the action of weather plays a major part in road performance; research on hardening was described.

There is at present considerable interest in the possibility of using bituminous materials in the construction of road bases. These materials combine the advantages of a bound material with a large measure of flexibility. Investigations are in progress in the Laboratory on the mixtures to be used, and experimental sections have been included in the full-scale experiments already discussed. The specifications at present recommended were shown.

On rolled asphalt wearing courses in which the stone content is 45 per cent or less, it is normal practice to lay a final application of coated chippings. To spread the chippings uniformly by hand requires skilled labour and slows up the work even where there is no shortage of skilled manual workers. A working model was shown of a machine now under development that spreads coated chippings at a predetermined rate. The machine consists of a hopper and rotating metering device carried on rollers which travel on the surface of the newly laid rolled asphalt. In the construction of new roads the spreader would be carried on rubber-tyred wheels on the concrete edge strip or on the base-course outside the mat being laid.

A group of exhibits illustrated the factors that affect the riding quality of a concrete road and the procedures needed to obtain good riding quality. Experiments undertaken by the Laboratory have shown that, when efficient spreading and compacting machines are used, the riding quality obtained is of a very high standard, the average irregularity index being 32 in. per mile with only 25 per cent of the values greater than 40 in. per mile. No significant differences in riding quality were obtained when different mixes were used, and different mix proportions did not appear to be important when modern power-propelled plant was used.

One of the problems investigated by the Laboratory is the possibility of using prestressed concrete for roads. Tests have been undertaken to determine the performance of thin sections of prestressed concrete. An exhibit showed the comparative costs of reinforced and prestressed concrete roads, work done in collaboration with the Belgian Centre de Recherches Routières, loading tests and measurement of subgrade restraint, and the development of a jack for prestressing slabs by cables with minimum interference with construction. In co-operation with the Belgian research organization a prestressed concrete road was built at Zwartburg, Belgium, in 1960. The experimental road is more than two miles long and includes prestressed, plain and reinforced concrete slabs. The performance of the road under traffic is being observed, and the possibility of carrying out loading tests will be considered at some future stage.

In recent years sections of road have been constructed with heating wires placed in the upper layer of the road structure to prevent the formation of ice

and frost, and to melt snow. A control device automatically switches on the heat when the road surface is cold and wet. The control device was shown in operation. The heating system has been installed in the underpass at Hook on the Kingston by-pass road (*A*.3) and in the one nearing completion at Hangar Lane, Ealing (*A*.40), on a bridge at Basildon, Essex, and on roads in Edinburgh, Slough and Harmondsworth. The Laboratory has also assisted with the design of the heating systems for many sections of road, including the Hammersmith fly-over, the Hyde Park Corner under-pass in London and for the approach ramps to the new Clyde tunnel in Glasgow.

Tropical Section

One of the main aims of the Tropical Section is to systematize knowledge of the engineering properties and occurrence of the soils and roadmaking materials in different territories. Exhibits illustrated: the nature and engineering properties of soils in individual territories and areas, including the use of aerial photography in terrain evaluation; work on tropical gravels, their use in road building and their suitability for stabilization with Portland cement and hydrated lime; stabilization of sands—full-scale experiment in Nigeria employing bitumen and Portland cement; tests on tropical aggregates; the relation between climate and moisture conditions under roads and runways; pavement design in the tropics.

The rapid growth of road traffic in developing countries is both a factor in the development and a symptom of it. Examples were shown of investigations in Uganda and North Borneo to determine the effects of providing roads on the rate and character of agricultural development. In the expanding towns and cities overseas there are already severe traffic problems and there is at the same time opportunity to plan the future growth of the towns so that the facilities required by future traffic are integrated with the needs of the community as a whole. Examples were shown of investigations undertaken in Hong Kong, Kingston (Jamaica) and Lagos to ease present traffic problems and to plan future needs.

Scottish Branch

The Scottish Branch of the Laboratory held open days during June 7–8 at Thorntonhall, Glasgow. The Branch was established in 1949 with the view of investigating problems of particular interest to Scotland, making known to all concerned with roads and traffic in Scotland the results of work undertaken at the parent Laboratory in England, and encouraging the application of the results of research.

While many of the exhibits displayed at Thorntonhall were those originally on view at Crowthorne and Harmondsworth, there were a number that illustrated the work undertaken in Scotland.

An investigation is being carried out on the effect of the new Forth road-bridge on the traffic patterns in the region. An exhibit described with photographs and maps an origin-and-destination survey carried out during the whole of one week in August 1960. Vehicles were stopped at three survey stations and more than 8,200 drivers, or about 43 per cent of the total, were questioned to obtain information on the traffic in the region before the opening of the new bridge. Analysis of the information obtained has not been completed, but preliminary results show that 22 per cent of the journeys start and terminate in Glasgow or Edinburgh, and about 34 per cent in five major cities.

The effect of new towns on the generation of traffic and on traffic patterns is being studied using East Kilbride as a 'guinea pig'. Such studies should assist planners in predicting facilities required for new towns, for example, the number of lock-up garages that should be provided. In East Kilbride there is one car for every three families.

There is a shortage of crushed stone in certain parts of Scotland, and the Laboratory has been examining the possibility of using morainic (glacial) gravels for the construction of road bases and surfacings. For the base, glacial gravels are mixed with cement to produce a 'lean concrete', for surfacings they are mixed with bitumen to provide a reasonably cheap and durable wearing course. Since 1956 many short sections of road containing glacial gravels—each with a different composition—have been laid in various parts of Scotland. The results so far are very promising.

Peat occurs in many regions in Scotland. This soft, water-filled, organic material is compressed when a road is constructed on it and this often results in an uneven road surface with a poor riding quality. A group of exhibits showed how the Laboratory is tackling the problem of building roads on peat.

OBITUARIES

Prof. A. W. P. Wolters

Prof. A. W. P. Wolters, who died recently, was the first holder of the chair of psychology in the University of Reading and played an outstanding part in dissolving academic distrust of his subject and achieving its recognition. His patience in handling the problems of its acceptance in the outside world was effective as well as exemplary. As deputy president, during 1936–38, and as president, during 1939–41, of the British Psychological Society, he took a leading part in the difficult transformation of a somewhat ungainly association into an incorporated body with specific professional, as well as scientific, aims. Wolters, too, was among the first to urge the opportunity and the need to employ people with a psychological training in the event of war. Unusual hardihood and a complete lack of aggressiveness enabled him to survive the official flood of waste-paper and committee proceedings which resulted. The idea that objective study of the human organism is not irrelevant to human welfare and efficiency thus gained some degree of official recognition in war and survived to spread effectively in peace.

Wolters learnt his psychology, both academic and practical, the hard way. As a fourteen-year-old pupil-teacher, he had charge of the mental defectives "who littered the enormous classes" of the Board School where he taught. Perhaps this experience stood him in good stead later on. His catholic intelligence responded to such education as came the way of a pupil-teacher, and in due course he

entered University College, Reading, where he was to live and work for almost the rest of his life. W. L. de Burgh, the professor of philosophy, gave him such scope as was available to introduce psychology, and the good judgment with which Wolters handled this admirable but hazardous opportunity ensured a much earlier start for an independent department and honours school at Reading than was possible in many other places. His quiet advocacy, too, prevailed among manifold claims and restricted resources and somehow saw to it that a specially designed laboratory was built. Here, in the years which followed the War, he built up a school of psychology modest in size, but the quality of which aroused the envy and admiration of his colleagues elsewhere. At the same time his self-effacing talents were devoted to his University—he became deputy vice-chancellor in 1947—then preparing for complete removal to Whiteknight's Park, and beset by the difficulties of post-War expansion. His moderation and his constructiveness in meeting these problems were indispensable.

Wolters's modesty and simplicity were inherent and free of contrivance, his perceptiveness unforced. His qualities stirred young people much, but himself only a little, to intellectual achievement. His main explicit contribution to psychology—an extension of Head's concept of *Schema* into the field of conceptual thinking—is an original contribution the full implications of which probably remain to be appreciated. But the inspiration of his teaching and the gentleness with which he deflated the pretentious will long be remembered and admired.

R. C. OLDFIELD

Prof. A. F. Kapustinsky

ANATOLY FEDOROVICH KAPUSTINSKY was born in Zhitomir on December 19, 1906, and died in Moscow on August 26, 1960. He studied chemistry in the University of Moscow, from which he graduated in 1929. His main field of research was in physical chemistry and thermodynamics. He was particularly interested in work on the energetics of chemical reactions, in the energy of the crystal lattice, in crystallochemistry and its application to geology. In 1933 he proposed the second principle of crystallo chemistry, namely, that the energy of the crystal with its attendant properties is determined by the number of ions, their radii, their valencies, and their degree of polarization. At the same time he proposed a simplified equation for the energy of the crystal lattice in ionic crystals, and in 1943 he produced a revised equation, which enabled him to calculate the energy of the crystal lattice of compounds with complex ions. Still later, he produced a number of equations applicable to various crystallochemical problems.

Besides these problems, Kapustinsky tried to solve many others, especially in thermodynamics. In collaboration with B. A. Shmelev, he devised a new method of physico-chemical analysis which enabled them to study complex equilibrium systems. He also published numerous papers dealing with thermochemical methods, with ionic compounds, electrolytes and chemical technology. By introducing the zero period into the Periodic Table he developed the idea of secondary periodicity which he linked with the periodic structure of atomic nuclei. This led him to discuss matter subjected to high pressure and then to apply these ideas to his new "Theory of the Earth" (see *Nature*, 180, 1245; 1957).

During his busy life, Kapustinsky occupied the following posts: professor of physical chemistry at the University of Gorky (1933–37); the same post at the Moscow Steel Institute (1937–41) and also at the University of Kazan (1941–43); professor of general and inorganic chemistry at the Chemical-Technological Institute in Moscow (1943–60). In 1939 he was elected corresponding member of the Academy of Sciences of the U.S.S.R.

Kapustinsky's publications are varied and numerous. Besides pure research, he published some thirty papers dealing with topics related to the history of chemistry. He also edited many books translated into Russian.

In private life Kapustinsky was a most jovial fellow and a very versatile conversationalist, and his death at the early age of fifty-four was deeply felt by his friends, of whom he had many.

S. I. TOMKEIEFF

NEWS and VIEWS

Applied Mathematics at Manchester : Dr. F. Ursell

THE Beyer professorship of applied mathematics at the University of Manchester is to be filled by Dr. F. Ursell from October 1, following the departure of Prof. M. J. Lighthill for the Royal Aircraft Establishment in 1959. Dr. Ursell is at present a lecturer in the Department of Applied Mathematics and Theoretical Physics, University of Cambridge. He took the Mathematics Tripos at Cambridge during the early years of the War (in company with his predecessor in the Beyer professorship), and spent the remainder of the War at the Admiralty Research Laboratory, Teddington. The introduction which he gained there to problems associated with water waves evidently made a deep impression, for he has pursued such problems steadily and successfully since that time, and has done much to revive interest in a 'classical' subject which—like so many others—was thought to be well understood and well tilled until war needs revealed deficiencies. In 1947, he was elected to a research fellowship at Trinity College, Cambridge, for a dissertation embodying his research on water waves. A short time later, he proceeded to the University of Manchester, where he held an I.C.I. Fellowship, returning to Cambridge to take up a lectureship in 1951. He was eventually elected to a research fellowship at King's College, and has received other tributes to his research, which is characterized by great analytical power. He is interested in a wide variety of mathematical techniques, and can be expected to maintain the high reputation of Manchester as a centre of teaching in applied mathematics.

Electrical Engineering at Birmingham : Prof. J. T. Allanson

MR. J. T. ALLANSON has been appointed to the recently established second chair in the Electrical Engineering Department in the University of Birm-

ingham. In this post he will be responsible primarily for the undergraduate work of the Department. Prof. Allanson entered the Department of Electrotechnics in the University of Manchester in 1938, taking his B.Sc. in 1942 and his M.Sc. in 1944. Although he had worked at the University on waveguides, he took for a year a post with Callenders Cables, Ltd., concerned with power cables and the cross-channel petrol pipe-line, and then went to Ultra Electric, Ltd., for two years to work on general electronics, including radio receivers. In 1947 he was appointed head of the Electrical Laboratory at the Telegraph Construction and Maintenance Co., Ltd., and was concerned once more with cables. In 1949 he was appointed lecturer in electrical engineering in the University of Birmingham, becoming senior lecturer in 1957. He is a man of very wide interests, embracing philosophy and biology as well as engineering; and, indeed, his strongest research interests lie in the field of nervous systems and particularly the mechanisms of hearing, and he has made some original contributions to this in co-operation with colleagues in the Department of Physiology. He has a strong natural interest in electrical net-work theory, and has published a number of papers on this topic, being particularly concerned with net-work synthesis. But his strongest enthusiasm is probably for teaching and students, and he has experimented in a number of ways to find better methods of teaching and to improve staff–student understanding and co-operation. He is also very interested in university administration. His appointment will give much satisfaction to his colleagues.

The Royal Scottish Museum, Edinburgh : Dr. Douglas A. Allan, C.B.E.

On September 15, Dr. Douglas A. Allan retires from the directorship of the Royal Scottish Museum, Edinburgh, the destinies of which he has controlled for nearly seventeen years. After graduating at Edinburgh he became assistant to the professor of geology in Edinburgh and afterwards was appointed lecturer in geology in Armstrong College (now King's College, University of Durham), Newcastle upon Tyne. Work on Lower Carboniferous geology in Fife earned him a Ph.D., and later on Lower Old Red Sandstone tectonics along the Highland Border a D.Sc. In 1929 he was appointed director of Liverpool City Museums. In 1945 he became director of the Royal Scottish Museum, and returned to his native Edinburgh to complete his active professional career. Under him, the Museum has played a much more prominent part both nationally and internationally, for he has served on the Joint Committee of the Carnegie United Kingdom Trust for twenty-five years, advising on the allocation and utilization of development grants, was chairman of the British National Committee of the International Council of Museums (Unesco) for seven years, and this year is president of the editorial board of *Museum* (Unesco). In 1952 he was director of the first Unesco seminar on "Museums and Education", held in New York. Under his directorship, the Royal Scottish Museum has been modernized in display techniques, has rendered wide service to research in many fields, and has embarked on schemes of periodical exhibitions of educational and cultural value. His early exploring adventures are reflected in his four years tenure of the presidency of the Royal Scottish Geographical Society and his official museum tours in North America, Asia and Central Africa.

Mr. Ian Robertson Finlay

Mr. Ian Finlay, who succeeds Dr. D. A. Allan, was born in New Zealand but educated in Edinburgh, graduating M.A. with honours in the history of art. He joined the staff of the Museum in 1932 and was successively assistant keeper and, since 1955, keeper of the Department of Art and Ethnography. He is particularly interested in display methods in all types of museums and will bring his undoubted talents to the front at an important time in the history of the Museum, which has practically completed its major overhaul since the Second World War. Mr. Finlay is secretary of the Royal Fine Arts Commission for Scotland, a Freeman of the City of London and a Member of Livery of the Worshipful Company of Goldsmiths, London. He has a strong interest in contemporary Scottish crafts and is a leading authority on Scottish gold and silver, having written several books and broadcast on numerous occasions. Last year he was awarded a Leadership grant by the United States Government to enable him to visit museums and galleries in North America.

Mr. Cyril Aldred

Mr. Cyril Aldred succeeds Mr. Finlay as keeper of the Department of Art and Ethnography of the Royal Scottish Museum. Born in London, he was educated at Sloan's School and entered later on a course of study in the History of Art at King's College, London, and the Courtauld Institute, graduating as a B.A. in 1936. He was appointed assistant keeper in the Department of Art and Ethnography in the Royal Scottish Museum in June 1937, and has served that Department with distinction ever since, except for one lengthy break with the Royal Air Force during the Second World War, and two years as associate curator in the Egyptian Department in the Metropolitan Museum of Art, New York. From an early stage he has taken a very strong interest in Egyptology and the life and arts of ancient Egypt, a field in which he now has an established reputation, and in which he has published a considerable amount of work, both on the monograph and the popular level. A competent practitioner himself in gold and silver craftwork, he is particularly well qualified to judge standards and methods in the works in his care, and brings a new attitude to the study of the ancient Egyptians to that more commonly brought by the philologists. He has already made a notable impact on museum techniques by his staging of exhibitions, both temporary and permanent, and in the latter, in Edinburgh, introduced the novel idea of employing contemporary writings to explain and illuminate his ancient Egyptian exhibits—rather than the 'hindsight' view of a twentieth-century savant.

Veterinary Anatomy at Liverpool : Prof. A. S. King

Dr. A. S. King, senior lecturer in veterinary anatomy in the University of Bristol, has been appointed professor of veterinary anatomy in the University of Liverpool. He is thirty-nine years of age, and was educated at Felsted School, Essex, and the Royal Veterinary College, London, where he obtained the degree of B.Sc.(Vet.Sci.) in 1946 and the degree of Ph.D. in 1956. During July 1945–December 1950 he held teaching appointments at the Royal Veterinary College, London. In January 1951 he was appointed lecturer in veterinary anatomy

in the University of Bristol, and senior lecturer in veterinary anatomy in 1960. During 1960 he held the post of visiting associate professor in anatomy in the School of Veterinary Medicine, Philadelphia. Dr. King has been a member of the consultant editorial board of *The Journal of Small Animal Practice* since its inception.

National Reference Library of Science and Invention

Aslib Proceedings for July (13, No. 7) includes a report of the discussion meeting on the National Reference Library of Science and Invention arranged in London on May 9, at which Prof. R. S. Hutton had urged the paramount importance of the potential users' requirements in planning the Library, and expressed his doubts as to the adequacy of present plans and intentions. Sir Frank Francis said that the British Museum would have preferred to have the new Library entirely on the Bloomsbury site, but use of the South Bank site would permit full operation by 1965, whereas development of the Bloomsbury site would not be completed until 1985. The National Library of Science and Invention would be one of a number of departmental libraries of the British Museum, the work of which would be thereby co-ordinated. There would be a separate science library in Bloomsbury devoted to the historical material; current material would be held on the South Bank. The new Library would be limited to 500,000 volumes because the appropriate committee of the Advisory Council on Scientific Policy thought this was the largest number compatible with open access. All material discarded from the National Reference Library would go to the British Museum collections. Research was needed on documentation services for scientists and provision had been made for this in the plans. It had been hoped to include rooms for lectures and exhibitions, but, in view of the limited space on the South Bank, these facilities and accommodation for investigations into the use of scientific literature would be provided in Bloomsbury. Investigations were being made to determine the reference services that were needed and the results would be made public. A general reference service would be provided at Bloomsbury. In the discussion the need for a first-class reference and information service was stressed, and also the importance of a rapid shuttle service between Bloomsbury and the South Bank, making full use of the latest possible techniques. Prof. Hutton said that his fears had only been partly dispelled: the fundamental importance of the Library was not grasped by policy makers in or near the Government.

The Role of University Extra-Mural Libraries in Great Britain

In *Library Association Pamphlet* No. 21, E. P. Pritchard reviews the aims, organization and methods of university extra-mural libraries in Great Britain and the results so far achieved (Pp. 32. London: The Library Association, 1961. 5*s*., and 3*s*. 6*d*. to Library Association members). This is a comparatively recent development, and in some areas library services for extra-mural courses have an air of improvization, although in others present needs are well on the way to being met. Mr. Pritchard visualizes, however, a considerable growth during the next fifteen years in this side of university work, largely through an increase in the number of extra-mural students who are graduates or have been in a sixth form. He believes the co-operation of extra-mural libraries, public libraries and the National Central Library, which already exists in several areas, could meet almost any foreseeable needs, provided: (1) that library services and the resources devoted to them receive the same emphasis in extra-mural work as in university internal teaching; (2) that each type of library makes the kind of contribution it is best fitted to make; and (3) that tutors never forget the importance of reading in adult education and lose no opportunity of applying this principle. Mr. Pritchard gives an admirably concise survey of various problems and difficulties encountered in practice and the methods of overcoming them.

Zoological Society of London : Annual Awards

The Zoological Society of London will, from 1962 onwards, make the following annual awards for contributions to zoology. These will be in addition to the Society's Gold, Silver and Bronze Medals which have been in existence since 1847.

The Scientific Medal of the Society, which was instituted in 1938, will be presented to a zoologist, less than forty-five years of age, for distinguished work in zoology. The Prince Philip Prize will be awarded to a pupil of zoology, on the basis of an account of some practical work in the field of animal ecology. This will be open for competition to all pupils of zoology in schools in Great Britain, Northern Ireland, the Channel Isles and the Isle of Man, who are studying for the General Certificate of Education A-level examination or an examination of equivalent standard. The award will be a sculpture in bronze, "Animal Head", by Mr. Henry Moore. The entries will be in the form of an essay of not more than 3,000 words, the first award being made in April 1962. Full details will be sent to schools in September of this year, but the regulations can, in the meantime, be obtained from the Zoological Society (Education Department), Regent's Park, London, N.W.1.

Two further awards for contributions to zoology, also sculptures by Henry Moore, have been made possible through the generosity of Granada Television Network. The Stamford Raffles Award, commemorating the founder of the Zoological Society, will be awarded to an amateur zoologist for contributions to zoology, or to a professional zoologist for contributions which extend beyond his field of specialization. This sculpture, "Animal Form", will first be awarded in April 1962. The Thomas Henry Huxley Award will be presented annually to a postgraduate research student in a university in Great Britain and Northern Ireland, for outstanding original work in zoology submitted as a doctor's thesis. This award will also be a sculpture, "Bird", the first award being made in April 1962. Mr. Henry Moore has generously agreed that a limited edition of each of the sculptures may be made, thus establishing the awards for some years. Thereafter the form of award will be reviewed.

Employment of Graduates : a Durham University Survey

The fifteenth annual report of the Durham University Appointments Board, and the third to be published, expresses the view that the demand for graduates of all kinds, from many different types of employers, is still rising, and, generally speaking, more Durham graduates and postgraduate students were seeking first appointments in 1960 than ever before (Fifteenth Annual Report. Pp. 9. Durham:

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Durham University Appointments Board, 1961). Fewer men seemed to be applying to the Atomic Energy Authority, but the Board did not notice the diminished interest in the heavy electrical plant industry found elsewhere. Of male graduates in science taking up paid employment, 13 per cent went straight into school teaching, compared with 12 per cent in 1959; for women, the corresponding figures were 67 per cent and 58 per cent; 50 per cent of the men graduating in arts and 70 per cent of the women went into teaching, and of these, 75 per cent attended teacher training courses. Comment is made on the very low present-day demand for geologists, the acute shortage of mathematicians (the proportion entering teaching has in consequence declined), the increased demand for arts men from industry and the increased recruitment to the public service, though public service overseas attracted only one recruit. The serious lack of information bearing on the proper employment of the woman arts graduate is stressed.

Vision Research

THE appearance of yet another scientific journal is not necessarily a cause for rejoicing, but Pergamon Press is certainly to be congratulated on its initiative in founding *Vision Research*, an international journal the first number of which appeared in June (1, Nos. 1/2; June 1961. Pp. iv + 201. Annual subscription rates: (*A*) for libraries, government establishments, research laboratories, etc., £10 (30 dollars); (*B*) for individuals who place their orders directly with the publisher and certify that the journal is for their personal use, 70*s*. (10 dollars). London and New York: Pergamon Press, 1961). Several different disciplines have an interest in the subject of vision—psychology, physiology, ophthalmology, physics, photo-chemistry, genetics—and the subject has applications in very many different fields. It is too much to hope that all papers on vision can be channelled into a single journal, but if the best of them find their way into *Vision Research* this will be an immense gain to visual scientists, who at present have to search through a great variety of journals. It will also enhance the prestige of the science itself and stimulate more rapid progress in the subject. The first number has been dedicated very fittingly to Commander Dean Farnsworth, who was the guiding spirit when the project was first under discussion, but who died while the plans were still in the formative stage. A board of editors, under the chairmanship of Dr. T. Shipley, of Miami, is supported by a large honorary editorial advisory board drawn from some twenty different countries and representing the leading workers in the field. Success in a venture of this kind depends most of all, perhaps, on maintaining a high standard in the quality of the papers that are accepted for publication and on the quality of the publication itself. The first number has got away to a good start on both counts.

National Museums of Southern Rhodesia

IN the annual report of the National Museums of Southern Rhodesia for 1960 reference is made to the planning that has been undertaken for the provision of three new museums buildings at Salisbury, Bulawayo and Umtali (Pp. 22. Bulawayo, Salisbury, and Umtali: National Museums of Southern Rhodesia, 1961). To further these important projects the Administrative Section of the organization was moved to Salisbury as being better placed strategically to cover the three units. The move also relieved the Administrative Section, at a time of intensive planning, of the detailed work of any one unit. There have been several changes in the staff, and Colonel H. L. Boultbee, curator of the Queen Victoria Museum, has completed his contract with the Trustees. Much research has been undertaken and members of the staff contribute to an impressive list of publications.

Maccabaean Prize and Medal

ENTRIES are now invited for the 1962 Maccabaean Prize of twenty-five guineas and a Bronze Medal, which is awarded annually by the Faculty of the History of Medicine and Pharmacy of the Worshipful Society of Apothecaries of London. Competitors, who must be less than thirty years of age on March 15, 1962, will be required to submit before that date an essay of not more than 6,000 words on any subject connected with the history of medicine or pharmacy. Further details can be obtained from the honorary secretary of the Faculty, Dr. F. N. L. Poynter, The Wellcome Historical Medical Library, The Wellcome Building, Euston Road, London, N.W.1.

Plastics Institute Lectures on "Plastics"

THE Plastics Institute is again arranging a series of lectures on "Plastics", to be held at the City of London College, Moorgate, in the autumn. The lectures are of an introductory nature and are intended for non-technical staff. Applications for registration forms should be sent to the City of London College as soon as possible. The programme is as follows: "Types of Plastics", by Dr. C. A. Redfarn (October 2); "Plastics and the Chemical Industry", by Dr. E. S. Narracott (October 9); "The Structure of the Plastics Industry", by C. J. G. Stanley (October 16); "Plastics as Replacement Materials", by K. B. Bartlett (October 23); "Creation of New Markets by Plastics Materials", by C. W. Welch (October 30); "Plastics and Packaging", by Dr. G. Swift (November 6); "The Plastics Industry and the Rubber Industry", by J. A. Rhys (November 13); "The Future", by Dr. V. E. Yarsley (November 20).

University News: Leeds

THE following appointments have been announced: Dr. P. Grosberg, to a research chair of textile engineering in the Department of Textile Industries; Dr. P. R. Brook and Dr. G. A. Morrison, to be lecturers in the Department of Organic Chemistry; Mr. R. S. Davidson, formerly demonstrator in the Department, to be Brotherton Research Fellow in the Department of Organic Chemistry; Dr. I. G. Gass, to be lecturer in the Department of Geology; Mr. W. Houghton-Evans, to be lecturer in architectural engineering in the Department of Civil Engineering; Mr. M. Kirk, at present lecturer in the Department of Geography, to be lecturer in the Department of Social Studies; Mr. P. Levi, to be Bernard Hickson Fellow in the Department of Botany; Dr. G. A. Salmon, to be J. W. Wootton Research Fellow in the Department of Chemistry; Dr. Jean E. Wilkinson, to be Medical Research Council Research Fellow in the Department of Medicine.

The following grants and donations, among others, have been received: £1,000 from the Yorkshire Council of the British Empire Cancer Campaign, for the Department of Physical Chemistry; £15,000 a year for seven years from the Anglo-American Corporation of South Africa, Ltd., to continue the work of the Research Institute of African Geology, and

£13,362 during two years from the Department of Scientific and Industrial Research for an investigation into African rocks, for the Department of Geology; £1,000 from Courtaulds, Ltd., for the Department of Textile Industries; £1,000 during two years from Messrs. W. L. Ingle, Ltd., for the Procter Department of Food and Leather Science; £2,470 from the Ministry of Aviation for a research project in the Department of Metallurgy, Houldsworth School of Applied Science; £2,897 during two years from the Department of Scientific and Industrial Research for a research project in the Department of Biochemistry.

London

THE following appointments have been announced: Prof. W. D. Gill, professor of geology and mineralogy in Trinity College, Dublin (see *Nature*, **172**, 1082; 1953), to the chair of oil technology tenable at the Imperial College of Science and Technology; Mr. S. B. Watkins, head of the Department of Chemical Engineering at King's College, to the University readership in chemical engineering tenable at that College; Dr. Brian Abel-Smith, lecturer in social science at the London School of Economics and Political Science, to the University readership in social administration tenable at that School; Mr. L. P. Foldes, lecturer at the London School of Economics and Political Science, to the University readership in economics tenable at that School; Mr. Harry Townsend, lecturer at the London School of Economics and Political Science, has been appointed to the University readership in economics tenable at that School.

The title of professor of civil engineering has been conferred on Mr. J. K. T. L. Nash, in respect of his post at King's College; that of professor of powder technology has been conferred on Dr. H. E. Rose, in respect of his post at King's College; that of reader in economics on Mr. J. Wiseman, in respect of his post at the London School of Economics and Political Science; that of reader in engineering science on Dr. J. M. Zarek, in respect of his post at King's College; that of reader in pure mathematics on Dr. K. W. Gruenberg, in respect of his post at Queen Mary College.

Sheffield

CONTRIBUTIONS to the University's Development Fund, the appeal for which was launched early in 1960, now total £480,975. While the major portion of the cost of the University's expansion is being borne by the Government, the University is required to make a contribution from its own funds towards the total allocation. It is to this end that the Fund will be used.

Announcements

H.M. THE QUEEN has been pleased to approve the grant of a Charter to the British Institution of Radio Engineers. The grant of the Charter is recognition of the vital part played by radio and electronic engineering in modern times.

MR. RONALD BERESFORD DEW has been appointed as visiting professor of industrial administration in the Faculty of Technology at the Manchester College of Science and Technology.

DR. R. M. LAWS, a principal scientific officer at the National Institute of Oceanography, Wormley, Surrey, has been appointed senior investigator in the Nuffield Unit of Tropical Animal Ecology, and will take up his duties at the Unit's field station at Mweya, Queen Elizabeth National Park, Uganda, in the autumn.

A SUMMER school on corrosion has been arranged by the Metallurgy Department, Battersea College of Technology, and will be held during September 25–28. The topic will be "Corrosion Problems in the Electrical Power Industry". The course has been arranged in conjunction with the Corrosion Group of the Society of Chemical Industry, and will be given by independent lecturers. Enrolment forms can be obtained from the Secretary (Summer School), Battersea College of Technology, London, S.W.11.

THE second conference on "Nuclear Reactor Chemistry" and the fifth conference on "Analytical Chemistry in Nuclear Reactor Technology" will be held in Gatlinburg, Tennessee, during October 10–12, under the sponsorship of Oak Ridge National Laboratory. Further information can be obtained from W. R. Grimes, Oak Ridge National Laboratory, P.O. Box X, Oak Ridge, Tennessee.

A SPECIAL meeting of the Iron and Steel Institute of Great Britain and the Metallurgical Society of the American Institute of Mining, Metallurgical, and Petroleum Engineers is to be held in the United States and in Canada, and at the Metallurgical Society's annual congress in Detroit, during October 17–November 8. Further information can be obtained from K. Headlam-Morley, the Iron and Steel Institute, 4 Grosvenor Gardens, London, S.W.1.

A SYMPOSIUM on "Corrosion in the Nuclear Energy Industry" is being organized by the Société de Chimie Industrielle, 28 rue Saint-Dominique, Paris (7e), and will be held during October 19–20. The symposium will be concerned specifically with corrosion in sheath materials.

A SYMPOSIUM on the freeze-drying of foodstuffs will be held at the Department of Chemistry and Food Technology, Borough Polytechnic, London, during October 19–20. The symposium will deal with the development of freeze-drying techniques, a survey of the commercial equipment at present available and a discussion of the potentialities of the technique in various branches of the food industry. Nutritional aspects will also be included. Pilot plant and model freeze-drying equipment, samples of freeze-dried products and packaging materials will be on display. Further details can be obtained from the Secretary, Borough Polytechnic, Borough Road, London, S.E.1.

AN interscience conference on "Antimicrobial Agents and Chemotherapy", sponsored by the American Society for Microbiology, is being held at the Hotel Commodore, New York City, during October 31–November 2. The first day will be devoted exclusively to infectious diseases, and the second and third to various aspects of antimicrobial agents, including their chemical evaluation. Further information can be obtained from the American Society for Microbiology, 19875 Mack Avenue, Detroit 36, Michigan.

THE Research Fund of the Chemical Society provides grants for the assistance of research in all branches of chemistry. Applications for grants should be sent to the General Secretary, Chemical Society, Burlington House, Piccadilly, London, W.1, not later than November 15.

THE BRITISH HYDROMECHANICS RESEARCH ASSOCIATION

THE Laboratory of the British Hydromechanics Research Association at Harlow was open to visitors during June 13–15, when several hundred representatives from industry and the academic world saw details of the research carried out.

The Association specializes in the industrial application of fluid flow and serves a very wide range of industries. Some recent problems investigated are: the ventilation of mines; the design of centrifugal pumps; water hammer in pipe lines; rotary and reciprocating seals; cavitation; the hydraulic transport of solids; friction losses in pipe fittings; and hydraulic design of civil engineering structures. These problems are typical of the extensive interests of the Association. The numerous and apparently unrelated difficulties which arise in such industrial applications of fluid flow often have a fundamental similarity, and for this reason can sometimes be solved through the experience of specialists in fluid mechanics. The Association has been building up such a team in the twelve years since its formation, and can now offer members a pool of such specialized knowledge. Despite the name 'Hydro' in the title, the Association is interested in all fluids, both gaseous and liquid, and its membership includes such diverse industries as hydraulic machinery, instruments, paper, chemicals, aircraft, oil, brewing and the nationalized industries of coal, electricity and transport.

In its research on centrifugal pumps, the Association aims at establishing the sources of loss within a pump, which should lead to a better understanding of the way in which the efficiency of the pump can be improved. This work involves investigations of the Reynolds number effects on pump performance, the relation between impeller and volute characteristics and the overall machine characteristic, and the measurement of disk friction loss. The exhibits demonstrated the refined instruments necessary to study the distribution of velocity and pressure within a rotating impeller. This approach to the problem of centrifugal pump research will incidentally assist in the establishment of acceptable laws for the scaling up of model-pump tests, and for the use of air tests for the prediction of water-pump performance.

Of additional interest to those involved in the testing of hydraulic machinery was an exhibit summarizing the variety of items which have been studied relating to the design of pump test-circuits. These have included the use of guide vanes to prevent swirl and the development of a cascade design which will be more rigid than conventional cascades, and less sensitive to upstream-flow conditions. A 'pepper-pot' valve has also been developed which has the dual advantages of good control characteristics and low loss when fully open. For any circumstances where it is required to produce a good velocity distribution upstream of a test pump or flow-measuring device, a 'flow straightener' has been designed. This combines the features of a graded resistance and a honeycomb, and will reduce considerably the extent of straight pipe required upstream of a pump or flow-meter. A new understanding of the conditions of flow within a shielded pitot tube has enabled a simple shield configuration to be devised, which renders the pitot tube insensitive to the direction of flow over an angle of about 40°.

The Association has been able to assist in the solution of several mixing problems, and an exhibit devoted to this subject illustrated several different aspects of mixing. One of these is a study of the mixing of a jet of water discharged into a shallow lake. The purpose of the research was to check that adequate mixing took place when power-station cooling water was discharged into a lake, so as to avoid the risk of warm water re-circulating into the intakes, and to reduce the danger to the fish population. Another application of techniques of mixing is the development of an in-line blending unit which successfully mixes, in given proportions, fluids of widely differing viscosities. This uses an orifice designed to have a discharge coefficient which does not vary with Reynolds number.

Two recent items of research were on show, which were of particular interest to makers and users of control valves. The incidence of cavitation in such valves introduces risk of material damage to the valve seat, and, by increasing the loss through the valve, interferes with its effective operation as a control unit. The exhibit reminded makers and users of the importance of checking for cavitation, and showed the importance of a dimensionless 'cavitation number' in defining the incidence of cavitation. Research is also being undertaken on the oscillation of relief valves, a frequent source of trouble in hydraulic engineering. The model valve on show demonstrated that unstable oscillations could occur at several frequencies, depending on the damping.

The water-driven vacuum pump is a device familiar to laboratory workers, but it may not be appreciated that the same technique is becoming more widely used as an economic means of maintaining vacuum in power stations and chemical plant. The essential design features of these pumps were demonstrated with the aid of a transparent model, which showed the importance of ensuring good mixing between the driving fluid and the entrained gas.

When air is released under water from a line source a rising vertical current is induced which, when it reaches the surface, divides into two surface water currents. The windward side current is capable of breaking approaching waves, so that relatively calm water is found in the lee side of such a 'pneumatic breakwater'.

At the request of a Government research establishment, the British Hydromechanics Research Association has studied the relationship between air flow and jet velocity, and has found means of accounting for the effects of slip of the air bubbles.

The exhibits relating to models of civil-engineering structures and pump sumps demonstrated the increasing use of models to ensure the achievement of design requirements, and to devise modifications which could lead to economies in head loss.

For spillways, the function of the model is primarily to study the most suitable design of stilling basin to dissipate the energy of the water at the bottom of the

structure. This aspect was illustrated by a model, at present being tested, of the spillway for the Sasuma Dam in Nairobi.

Models of the cooling-water intakes for the Sizewell nuclear power station were also on show. One, an air model, was being used to determine the head losses at the junctions between the intake shafts and tunnels. As a result of the tests made on this model, modifications to the hydraulic design which naturally had to be compatible with the structural engineering requirements have reduced the head losses due to the junctions by 40 per cent.

The diversity of interests of the Association was of advantage in this case, as the modifications were based on experience gained previously in the study of ventilation schemes of mines. These intakes will be located in a tidal estuary, and a water model has been constructed to examine the effects of tidal flow and tide-level on the flow and head loss in the intakes, and the possibilities of the formation of air entraining vortices.

The Association is also active in the rapidly expanding field of 'industrial hydraulics'. There are many problems involved in the design and utilization of high-pressure oil hydraulic machinery, and a particular example is the use of slipper pads to transmit the thrust from a swash plate to the pistons in an axial-piston pump. An analysis of the conditions for minimum power consumption of these slipper bearings has shown that the minimum occurs when the viscous drag loss is three times as large as the flow loss, although this ideal cannot always be attained as the resulting film gap would be too small. A new apparatus has been built to test slipper bearings under dynamic conditions.

Examples of machines embodying design features of this nature were seen in the form of two experimental power transmissions. One is for applications which require a rapidly variable speed, and is a 2 horse-power ball-piston pump/motor machine. The extremely low inertia of this unit is demonstrated by the fact that full reversal of rotation at 3,000 r.p.m. takes about 0·01 sec. The second exhibit was designed for a motor-vehicle drive where a prime requirement is high efficiency. This unit employs a differentially coupled pump and motor and has an efficiency exceeding 90 per cent, over a wide range of speed.

The Association has been active in work to ensure compatibility between a hydraulic system, and the hydraulic fluid. A rig was demonstrated for the evaluation of hydraulic brake fluids to study, over a wide range of temperatures, such factors as brake response, leakage, seal life and fluid deterioration. A committee has recently been formed to draw up a specification which will define adequately the properties required of a fluid to be used in particular applications.

Recently, the efforts of the Association in research on fluid sealing have been increased considerably by special contributions from members. This research is directed towards studying the physical processes involved in sealing in mechanical seals, stuffing boxes, and reciprocating shaft seals. A new test rig has been built to study the behaviour of mechanical seals at pressures up to 3,000 p.s.i. In particular, the rig will be used to investigate the possibility that the present limits on the pressure rating of this type of seal are due to mechanical distortion.

In reciprocating-shaft seals it has been found that the leakage is proportional to the square of the shaft speed, and independent of pressures above 1,000 p.s.i. The leakage increases and the friction decreases as the fluid viscosity is increased.

Although the exhibition was primarily of the present research work, an equally important function of the Association is to assist members in the solution of day-to-day problems involving fluid flow, and in issuing an *Abstract Bulletin* and technical reports which keep the membership in touch with the work carried out in other laboratories.

A problem common to all research associations is that of ensuring that the designers and development engineers, who will benefit most from the experience available at the laboratory, are kept informed of the service which is available. To this end, a mobile unit has been fitted out with exhibits demonstrating all the aspects of the work of the Association. The visits made so far by the unit, to member firms, have resulted in a fruitful interchange of ideas between the research staff of the Association and their colleagues in industry. S. T. Bonnington

BLOOD GROUPS OF ANIMALS OTHER THAN MAN

A CONFERENCE on "Blood Groups in Infrahuman Species", organized by the New York Academy of Sciences, was held in New York during May 15–16. The programme of papers arranged by Drs. D. B. Amos and C. Choen very fully covered much present-day research on the identification of blood groups in animals other than man, and their use in a variety of pure and applied sciences. On the first day several papers described work with animals useful in medical research. Drs. R. D. Owen (California Institute of Technology) and D. R. Anderson (School of Aviation Medicine, Brooks Air Force Base, Texas) had collaborated on a study of blood groups in the rhesus monkey, with the object of providing markers for transplanted erythropoietic tissue in irradiated animals. Blood group systems in the dog had been thoroughly investigated by Drs. S. N. Swisher, L. E. Young and N. Trabold (Rochester School of Medicine, N.Y.), and used in experimentation of clinical interest. The rabbit is valuable in comparative research on hæmolytic disease of the new-born, and for this a knowledge of its blood groups was an essential pre-requisite. Dr. C. Cohen (Battelle Memorial Institute, Columbus, Ohio) pointed this out in his review paper, while making an eloquent plea for research workers to co-operate more closely in establishing nomenclature of blood group systems. Dr. Cohen also provided an illustration of the wider interest of the conference with his discussion of genetical aspects and natural selection. The usefulness of rats as laboratory animals was exemplified by the inclusion of three papers about them. Dr. R. D. Owen reported on genetic systems of antigens which had proved abun-

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dantly useful as tools in a variety of experimental studies, in particular of red cell chimærism following either parabiosis, embryonic injection or bone marrow injection after X-irradiation. The interest of Drs. A. E. Bogden and P. M. Aptekman (Biochemical Research Foundation, Newark, Delaware) was in inherited hæmagglutinogens of rats and their relationship to histocompatability factors. The presence or absence of one hæmagglutinogen determined growth or rejection of a tumour, and this system showed a number of other interesting parallels with the *H*-2 factors of mice. In a careful review paper, Dr. J. Palm (Wistar Institute, Philadelphia), described her own work with blood groups and histocompatibility factors of rats and related it to that of other investigators.

The first of three papers on mice was a review by Dr. D. B. Amos (Roswell Park Memorial Institute, Buffalo) in which most emphasis was naturally enough placed on the classical *H*-2 system of tissue and red cell antigens. A clear account of the serological and genetical complexity of this system was given. Drs. R. Basch and C. A. Stetson (Bellevue Medical Center, New York) followed with a description of results obtained using an assay method for those antigens of mice which elicit cytotoxin and hæmagglutinin production (antigens which they believe to be absolutely identical). A wide range of isoantigen activity—as judged by ability to absorb or neutralize cytotoxins—was found among various mouse tissues. Further detailed chemical results included the important finding that in liver homogenates antigenic activity was concentrated in the lysosome-rich fraction. Finally with mice, Dr. J. W. Goodman (Oak Ridge National Laboratory) described experiments in which blood or bone marrow, or both, were injected into irradiated mice, and the fate or establishment of the donor-type cells followed by serological means, either hæmolytic, for red cells, or cytotoxic, for lymphoid cells.

The remainder of the papers on the first day were of more general zoological interest, rather than specifically medical. Dr. J. Buettner-Janusch (Department of Anthropology, Yale) had studied the reactions of the red cells of various primates with various antisera prepared to agglutinate human *A* or *B* red cells. This work was intended primarily as an aid to anthropological investigations. In connexion with an entirely different sort of population study Dr. G. F. Ridgway (U.S. Bureau of Fisheries, Seattle) gave a fascinating account of the extremely difficult work involved in analysing blood groups of salmon and trout, primarily using isoimmunizations. Dr. W. H. Hildemann (University of California at Los Angeles) interpreted the term 'blood groups' in its very widest sense in considering the literature and his own work on genetic specificities of amphibians and reptiles. Individual differences in red cell antigens have been found in only five species; no intraspecific differences in serum proteins—as determined electrophoretically—have yet been found, although their existence as raw material for speciation must be postulated, in view of the known ordinal, familial or interspecific differences. In contrast to the above sparse evidence of diversity, tissue transplantation studies yielded evidence of a high degree of intraspecific antigenic diversity. The role of individual red cell antigens in speciation has of course been the main theme of the classical researches of Dr. M. R. Irwin *et al.* on pigeons and doves, and an authoritative account of this work was presented by Dr. D. H. Shaw (University of Wisconsin, Madison). Other aspects mentioned included the study of hybrid substances, and the use of blood-group factors as markers in somatic mutation work and in radiation chimæra research (the latter being Dr. Shaw's own particular contribution). The final paper on the first day was introduced humorously by the chairman as being about blood groups of bacteria. Dr. G. F. Springer (University of Pennsylvania, Philadelphia) had continued his previous work on the immune origin of so-called natural anti-human *B* antibodies —in which 'germ-free' chicks were inoculated with *E. coli* O_{86}—by studying the chemistry of the bacterial substances responsible for the cross-reactivity. Among various findings were striking resemblances to human blood group substances, as shown by the composition of the carbohydrates produced by hydrolysis.

The second day's programme was devoted almost entirely to blood-groups of animals of agricultural interest. The first of four papers on chickens was a general survey by Dr. D. G. Gilmour (School of Agriculture, Cambridge, England). One point especially emphasized was that the chicken has particular advantages as an experimental animal for the study of selective factors influencing the polymorphism of blood groups. Considerable evidence on this aspect was already available. Dr. W. E. Briles (De Kalb Agricultural Association, De Kalb, Illinois) followed with a meticulous account of experimental techniques leading to the discovery of further blood group systems in chickens, of which six were described, five probably new. Evidence on the relationship between *B* locus blood group genotypes of female parents, and the economic performance of their 'hybrid' offspring (four-way crosses of inbred lines), was presented by C. P. Allen (Hy-Line Poultry Farms, Johnston, Iowa). This paper was especially interesting, being indicative of the possibility of the application of blood groups in some systems of commercial poultry breeding. Work on a red cell agglutinogen of chickens identified by certain plant seed extracts was described by Drs. S. L. Scheinberg and R. P. Reckel (U.S. Department of Agriculture, Beltsville). The occurrence of this factor is both inherited and influenced by hormonal state, occurring in genetically competent females only on the onset of egg-laying. Pigs were the subject of two papers, the first being a very clear account of the state of knowledge of their blood groups by Dr. E. Andresen (Iowa State University, Ames), much of whose own work had been done at the Royal Veterinary and Agricultural College, Copenhagen. His interest was primarily in genetic studies, and to a lesser degree in hæmolytic disease of the new-born. This condition was the prime interest in the second paper on pigs by Drs. R. Saison and D. G. Ingram (Ontario Agricultural College, Guelph), who reported on some systems previously described, and other new ones. They also briefly mentioned studies of blood groups of mink.

The report on horse blood groups by Dr. D. Franks (Department of Pathology, Cambridge, England) was largely concerned with hæmolytic disease of the new-born foal, and included much important material of veterinary and comparative medical interest. Dr. Franks's suggested interpretation of some of his serological findings—variation in amount of antigens in different genotypes—as being due to a *cis-trans* position effect provoked one of the most lively discussions of the meeting.

The last afternoon session was opened by Dr. C. Stormont (University of California at Davis), who

presented a highly competent and scholarly review of blood groups in cattle. In one cattle system, *J*, the antigenic substance is found in soluble form in various body fluids and tissues, and is absorbed on the red cells. This was the subject of a contribution by Dr. W. H. Stone (University of Wisconsin, Madison), who discussed physiological, genetical and chemical aspects. Dr. J. Gasparski (Polish Academy of Science) was unfortunately unable to attend the meeting, and his paper—on red cell antigens of the European bison identified by cattle blood typing reagents—was read for him. A definitive account of blood-group systems of sheep was given by Dr. B. A. Rasmusen (University of Illinois, Urbana), and the meeting concluded with a fascinating account, presented by Dr. A. Eyquem (Pasteur Institute, Paris), of blood group investigations by himself and Drs. L. Podliachouk and P. Millot. Species involved were chimpanzees, horses, mules, donkeys, sheep, pigs and cattle.

The papers described above are to be published as soon as possible in the *Annals of the New York Academy of Sciences*, and should remain definitive for some time to come. In addition, the meeting provided an excellent opportunity for exchange of opinions between workers in widely diverse fields, and there can have been few attending who did not acquire ideas for new approaches. Discussions were especially enlivened by the presence of some guests noted particularly for their research on human blood groups, among whom may be especially mentioned Drs. W. C. Boyd, S. D. Lawler, P. A. Levine and A. S. Wiener. Other activities associated with the meeting included an evening discussion meeting, which resolved to form an Immunogenetics Society of America, another evening discussion on blood-group nomenclature, and a most enjoyable dinner in honour of Dr. M. R. Irwin, arranged by some of his past students.

D. G. Gilmour

DESIGN AND USE OF RESPIRATORS

A JOINT meeting of the Ergonomics Research Society and the British Occupational Hygiene Society at Porton on July 5 and 6 was devoted to the study of respirators. The Societies were welcomed by Dr. E. A. Perren, director of the Chemical Defence Experimental Establishment (C.D.E.E.). The first paper, by C. G. Trotman (C.D.E.E.), dealt with the history of the subject. Dust masks were much the oldest form of respirator but were not really significant until a performance standard for industrial dusts was established in 1933. Canister respirators for protection against industrial gases grew from the chlorine box respirator of the First World War. The Chemical Works Regulations of 1922 enforced the provision of respirators of an adequate standard; since then, systems of coding and testing respirators for practically all known industrial and agricultural hazards have been laid down.

R. G. Dorman (C.D.E.E.) discussed fibrous filters for protection against aerosol particles. Sizes near 0·3μ diameter are the most difficult to filter; collisions with filter fibres are less probable for particles having low inertia and small Brownian motion so that they tend to penetrate the filter relatively easily. Experimental demonstration of a minimum of filtration efficiency at a certain particle size proved the general validity of the theory of filter action. The resin–wool filter was described, which combined extremely low air-flow resistance with high efficiency, due to electrostatic action. Methylene blue and sodium aerosol testing, with flame photometer detection, were also discussed, and desirable standards of protection indicated.

F. A. P. Maggs (C.D.E.E.) gave an account of the removal of gases by activated charcoal. Adsorption is a matter of the surface area of the charcoal and the number of molecules of gas, so that a high molecular-weight gas is retained better than one having small molecules. The life-time of a respirator against volatile, low-molecular-weight vapours may be quite short and there is some difficulty about giving proper warning of incipient failure to the user.

J. E. Cotes (Medical Research Council Pneumoconiosis Research Unit) dealt with physiological aspects of respirator design, including breathing apparatus supplied with oxygen. Factors of importance included dead space, flow-resistance, vision, fit of face-piece, and leakage. In addition to protecting the wearer, breathing sets with closed circuit made it possible to help him in situations involving physiological stress by humidifying, cooling and adding oxygen to the air which he breathed.

The ergonomics of the respirator was dealt with by R. J. Shephard (C.D.E.E.), who described methods of assessing the loss of performance resulting from wearing respirators. Deterioration of specific functions was slight, and the expenditure of energy occasioned by dead space and resistance only amounted to a few per cent of maximum effort under average conditions. General discomfort, sweating and unusual activity, however, reduced tolerance unless the subject were adequately motivated.

The work of ventilation was analysed by E. A. Cooper (Department of Anæsthesia, Newcastle upon Tyne), who directed attention to the contrasting resistance laws which were relevant to laminar flow in filter beds and turbulent flow in pipes and valves; the latter rises much more rapidly with an increase in the velocity of breathing. By considering the mechanism of respiration and cardiorespiratory function it was possible to lay down allowable standards of resistance.

Respirators, besides being protective devices, have been used in physiological research since the days of Lavoisier. H. S. Wolff (Medical Research Council, Hampstead) dealt with such applications. Mouthpieces were often used in place of masks, for brief experiments, but were uncomfortable and promoted salivation. Masks were important for measuring expenditure of energy from consumption of oxygen; types produced for anæsthetics, for airmen and for gas-masks were commonly adapted for physiological research, and various measuring devices could be attached to them.

Communication between persons wearing breathing equipment was the subject of a paper by J. Ernsting and J. E. Gabb (Royal Air Force Institute of Aviation Medicine). The situation is complicated in aircraft by the effects of reduced pressure on auditory acuity. Speech gives rise to respiratory flow patterns which impose a severe strain on the flow capacity of respiratory equipment.

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J. R. Carr (Royal Navy) spoke of the design of underwater breathing apparatus. The lungs of the user must be pressurized to the equivalent of the surrounding water, which results in a rapid increase with depth of the mass of gas consumed. Long endurance therefore necessitates either re-breathing or a surface supply of gas. Gases under pressure may affect man adversely, and psychological and physiological hazards may be introduced by limitations of the breathing set.

F. Lavenne and P. Leyh (Institut d'Hygiene des Mines, Hasselt, Belgium) compared the performance of two closed-circuit breathing sets, one working with compressed oxygen and the other with liquid air, and an open-circuit apparatus supplied with compressed air. Exercises performed at different temperatures showed that the inspired air temperature depended mainly on release of heat by absorption of carbon dioxide and was lowest in the open circuit apparatus. Light weight was an important point.

Industrial gas and dust masks were analysed by H. J. R. Letts (C.D.E.E.), who emphasized the influence on design of legislation requiring approved types. Dangers due to limitations of the absorbing medium are not serious, but the risk from leakage at the face-piece is significant and difficult to measure; a 10 per cent leakage due to physical activity was common, and wearability of a mask was an important attribute because of the user's tendency to avoid discomfort by discarding it. Leakage through the face-piece seems to be a fundamental limitation of the container respirator and may have to be avoided by electrically driven individual fan units.

The iron and steel industry uses respirators to a large extent, as was explained by J. Graham Jones (Richard Thomas and Baldwin, Ltd.). Major problems were carbon monoxide, from blast furnaces, and dust. For the former, only air-line masks had been successful, and compressed-air points were provided on furnace platforms. In the Fire Service, F. C. S. Shirling (Home Office) stated that respirators were useless, because of oxygen deficiency, and self-contained breathing sets were used which had a useful life up to 1 hr. An overriding factor in design was the necessity for instant availability.

The final session was conducted with Mr. A. C. Peacock (formerly of C.D.E.E.) in the chair, who also read a paper on the manufacture and testing of respirators. He suggested that it would be advantageous if users were to collaborate in producing a limited number of designs, which should be manufactured by a single organization.

S. T. Hermiston, R. F. Hounam and R. P. Rowlands (United Kingdom Atomic Energy Authority) listed the various types of apparatus for protecting people who handled radioactive substances. The problems encountered in other applications are here augmented by the necessity of decontaminating the appliances after use. For this reason, a worker is issued with a respirator from a pool instead of its being fitted to him individually, a practice which drew some criticisms.

The final application of respiratory devices discussed was the important one of mine rescue, by C. R. Senneck (Safety in Mines Research Establishment). A new closed-circuit liquid-oxygen set has outstanding performance; it weighs 33 lb. fully charged and gives cool dry air for more than 2 hr. During heavy work at 90° F., respiratory cooling of more than 1 kcal./min. is available and the resistance is only 1 cm. water at 300 l./min.; operation is automatic.

C. N. Davies

FOUR DECADES OF STATE FORESTRY IN GREAT BRITAIN

THE publication of the forty-first annual report of the Forestry Commission in Great Britain for the year ended September 30, 1960,* marks the first year of State forestry activity in its fifth decade of existence. In temperate countries it takes more than four decades to create new forests, a process which does not end with the planting of so many trees on so many acres. In the year 1959/60 the State planted 61,700 acres and private enterprise 36,900 acres, making a total of 98,600 acres. If this rate continues and if we assume that the average length of rotation, making allowance for the slow growth to be expected on much of the land being planted, is 80 years, then it would seem that the final wooded area will, in that time, amount to 8,000,000 acres plus 2,000,000 acres already existing, or 10,000,000 acres in all. If this is the avowed objective, the position at the moment is reasonably good. The competition for land in Great Britain has not prevented the Forestry Commission from adding during the year some 60,000 acres of plantable land to bring its total holding thereof to 1,618,400 acres. In describing the year's work it is interesting to find, however, that private forestry is placed before State forestry. This may be merely politeness, but things have certainly moved a long way since the last chairman expressed the view that private enterprise in forestry was doomed. It has greatly strengthened its position in recent years and the policy of paying grants in aid to the tune of £1,150,000 a year has given it a remarkable boost. The dedication of woodlands to productive forestry has advanced to the extent that the area of private woodlands eligible, or about to become eligible, for State aid is now 837,476 acres. There is thus quite a healthy balance between the managed woodlands of the State and those of the private owner. It is surely time to abolish, if possible, the needless complication of having two bases of dedication, for only two of the 562 schemes in operation are on basis II.

The report introduces to the public the concept of the 'working-plan', which is, in fact, a plan of management for the forest. It is defined as being "in effect, a blueprint for a forest or group of forests" and "a direction to succeeding generations of foresters ensuring that in all stages of development the basic objectives are kept clearly in mind". One might ask what is a blueprint? Have British foresters become that mechanically minded? In 1960 a new

* Forestry Commission. Forty-first Annual Report of the Forestry Commissioners for the Year ended 30th September, 1960. Pp. 69+9 plates. (London: H.M. Stationery Office, 1961.) 5s. net.

working-plan code came into operation. The prescribed format contains no fewer than 43 chapters, and, if the code is to be rigorously applied, it would appear that the technical staff will have even less time to undertake essential technical operations and may even be helping to dig its own grave.

So far, the Forestry Commission has been able to sell all its felled material, which amounted in 1959–60 to 20·1 million hoppus feet from a total area of forest of 1,283,256 acres. This presumably included the 500,000 hoppus feet blown down, equivalent roughly to the growth on 10,000 acres. While the problem of marketing is easy in those forests near industrial centres it will become acute in the next decade or two in the more remote forests, the location of which can readily be seen from an examination of the maps provided with the report. Improved methods of exploitation and transport are receiving attention and may be the solution, involving greater use of machines. Too much machinery used in forestry operations will kill employment in the country and increase it in the towns, however, which runs counter to a policy of re-population of the former. The wide scatter of the State forestry operations can be seen from the maps mentioned. This adds greatly to the risk of fire. The cost of fire protection is a heavy item in the creation from scratch of pure coniferous forests and £273,000 was spent thereon in 1960. Out of a total area of 1,618,400 acres of forest, only 1,596 acres, or 0·1 per cent, were lost by fire, the greater part through an excess of zeal.

A paragraph describes what has been done to preserve the remnants of the natural pine forests which are owned by the Forestry Commission. It mentions "a broad belt of the eastern Highlands" where the native Scots pine is a dominant species. The meaning of this is obscure. It is claimed that investigations began in the early days of the Commission into methods of treatment of the natural stands it had acquired. Details are not given, but the investigations were of the most meagre description and the main method of treatment has unfortunately been to plant up the greater part of these areas with introduced exotic conifers. The attention being devoted to other important indigenous species, especially oak and birch, is woefully inadequate, even in the research programme.

Forest research is the subject of a separate annual report. A relatively large staff is engaged in this work but its organization is far from satisfactory. It has engaged in numerous projects, many of which seem to be of minor significance. The main aim of research should undoubtedly be the improvement of sylviculture. This remark owes its origin to a statement in the report that fertilizers have been applied from the air to checked spruce stands in Cornwall, with impressive results. For how long will these results last and what was the cost? Much research work owes its origin to faulty sylviculture, and in the improvement in their sylviculture British foresters have a great deal to learn. Forestry in Britain has suffered from a lack of trained personnel of the higher ranks and still has a long and arduous task before it is to realize its main objectives.

Regarding the financial cost to Great Britain of the establishment and working of the Forestry Commission, this is an appropriate year in which to consider this, because, when the Commission was set up in 1919, it was claimed that it would be self-supporting in forty years' time. The total net sum spent since the start, after allowing for income other than Treasury grants, is £118,219,420, on which presumably some rate of interest is charged. Far from being now self-supporting, in 1960 payments were £13,930,162, of which only £3,130,639 was met by operational income from sales, etc. A Parliamentary vote of an additional £10,702,000 was still required. There is a somewhat naïve explanation of why forestry is not turning out to be the "counter to depopulation in the uplands" that it has for long been claimed to be. Almost any other industry could justify its existence by claiming that it gives indirect employment in other industries.

The manner of presentation of the Forestry Commission report is odd. The author of it is presumably the secretary of that body, but the report is signed by ten other persons and is presented to two Ministers in conformity with legislative requirements. To what extent each of the eleven persons in question takes responsibility for the contents remains uncertain. There is no minority report. Whether the two Ministers who receive it sit down and discuss it in detail, or whether each deals with it in turn, is doubtful. It should be mentioned, however, that the Forestry Commissioners can rely on advice from national committees, regional advisory committees, a Home-grown Timber Advisory Committee, a Committee on the Utilization of Home-grown Timber, a Mechanical Development Committee and an Advisory Committee on Forest Research. In all, between 150 and 200 persons are named in the report as having some say in the running of British forestry. This restricts the number of competent independent reviewers of their activities to a very few and makes their task invidious

M. L. ANDERSON

POTATO VIRUS DISEASES

ALTHOUGH conferences, especially those restricted to a particular subject (therefore small) can be very useful for providing opportunities for exchanging views, their proceedings are often superfluous. Most of the interesting information they contain has already appeared in scientific journals or in reviews as well. Recently, it has become customary to include at the end of each paper the discussion which it has stimulated, but most of it is trifling or irrelevant to the paper.

This criticism applies to the proceedings of the fourth conference on potato virus diseases, held at Braunschweig, during September 12–17, 1960*. As it is only three years since the last conference, it is not surprising that the twenty-three papers (175 pages) it contains have very few advances to report.

Recently, Dr. Stegwee discovered that potato leaf roll virus multiplies in the vector *Myzus persicae* and has now reported more evidence for this. Of special interest is the finding of P. Ehrhardt that

* Biologische Bundesanstalt für Land- und Forstwirtschaft, Braunschweig. Proceedings of the Fourth Conference on Potato Virus Diseases, Braunschweig, 12–17 September, 1960. Edited by J. Brandes, R. Bartels, V. Völk and C. Wetter. Pp. 175. (Wageningen: H. Veenman and Zonen, N.V., 1961.)

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infected aphids show significant decrease in oxygen consumption. V. Moericke discusses some electron micrographs of the salivary glands of *M. persicae*. (Unfortunately the micrographs are not reproduced.) Those from infected aphids contain rod-shaped particles (50 × 200 mμ) which are suspected to have some connexion with leaf roll virus if they are not the virus particles themselves.

The papers by C. Wetter and J. Brandes report some recent work at the Biologische Bundesanstalt, Braunschweig, Germany, which increases our scope for grouping viruses on serological relationships. Using Freund's adjuvant, antisera to viruses, having rod-shaped particles, were produced with precipitation titres of up to 1 : 500,000 (unusually high titres for viruses). With such antisera it has been possible to demonstrate serological relationships between viruses previously considered unrelated. Even more unexpected is the relationship found between viruses differing in particle-length such as between potato virus *X* (520 mμ) and white clover mosaic virus (480 mμ).

There are five papers on the resistance of potato varieties to virus infection, the inheritance of resistance and some practical aspects in testing for resistance. However, there is only one paper on field experiments. This may be the fault of the selection committee and not that this important kind of work is lacking.

In recent years soil-borne viruses are in the mode. In this respect the *Proceedings* are up to date, but of the several viruses discussed only potato rattle virus is appropriate to the conference. From the three papers attempting to review "some aspects" of virus research none contains anything new which has not been previously discussed in other review articles.

B. KASSANIS

CHROMOSOMES OF *NEUROSPORA*

RESEARCH workers at the University of Texas are investigating the genetic and nutritional simplicity of the red bread mould, *Neurospora*, to map the locations, on particular chromosomes, of the determinants of biochemical reactions by which particular substrates are metabolized for utilization in life processes. The work is expected to lead to a clearer definition of the gene.

The red bread mould *Neurospora* has long been regarded as one of the most useful of genetical experimental organisms. Pure strains can be multiplied a million-fold in a few days without genetic change. Each cell nucleus contains only a single set of genes, so recessive characters are not hidden by dominants. *Neurospora* can be grown in pure culture on a chemically known medium containing only sugar and a vitamin B (biotin) plus a few inorganic substances. In the natural state, the mould possesses the biochemical apparatus to make, from these simple materials, all the other constituents of its cells. Thus when a genetic element is disrupted experimentally by irradiation, the mutation may be expressed and mapped in terms of the measurable changes which occur in the simple nutritional requirements of the organism.

A large group of such nutritional mutations of *Neurospora* have been described by R. P. Wagner, C. E. Somers and A. Bergquist at the University of Texas in Austin. Plotting the distribution of the mutations on a map of *Neurospora* chromosomes, Wagner's group traced them all to a limited area of the fifth chromosome. Wagner's report is preliminary, but appears to record the first demonstration, in any organism with a single or double chromosome complement, of a direct relationship between one small segment of a single chromosome and a particular set of biochemical reactions. (Damage in the segment of chromosome affected directly the transformation of precursors of two important amino-acids—isoleucine and valine.)

The results also indicate a linear arrangement of the chromosomal material, with its active elements arranged to correspond to the order of sequence of the biochemical reactions it controls.

Wagner's work is supported by the Division of General Medical Sciences and the National Cancer Institute of the U.S. Department of Health, Education and Welfare. The publication *Highlights of Research Progress in General Medical Sciences*, 1960, presents a summary of Wagner's work and that of other investigators who are sponsored by the Division*.

* United States Department of Health, Education and Welfare, Public Health Service Publication No. 815: *Highlights of Research Progress in General Medical Sciences*, 1960. Items of Interest on Research Studies and Research Training Programs supported by the Division of General Medical Sciences, National Institutes of Health, Bethesda 14, Md. Pp. v+45. (Washington, D.C.: Government Printing Office, 1961.) 25 cents.

A CONCEPT OF ATOMIC EXISTENCE

By PROF. W. H. WATSON
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IN classical terms we have a manageable concept of physical existence. We represent things in a space-time map. Usually the processes by which recognizing, naming and surveying are accomplished are not shown in this map. Nevertheless, they could be, and whenever there is doubt or unclarity in their application, they are so represented for the purpose of clarifying representation. So long as the precision of representation does not approach the limits set by the Uncertainty Principle, all the apparatus and procedure just referred to can be used. We have confidence in ordinary language and rely on the training of physicists needed for applying it successfully in this context. The force of this consideration is illustrated in the skilful elaboration of classical explanation by Thirring in the first chapter of his *Principles of Quantum Electrodynamics*.

At the microphysical level, of course, there are physical impediments to applying ideas that we take for granted in the unformalized improvisation neces-

sary in using classical mechanics. The purpose of this brief article is to expose one aspect of our thinking that can be liberated from the pre-suppositions inherent in our forms of speaking about physical representation.

The classical picture of radiation misleads us with a picture of 'something' coming out of the source like a bullet from a gun. But to particularize the thing a label cannot be used; to name it one must point to the events connected by it.

For example, we know that the creation of a β-particle by a nucleus is associated with a detectable event—the recoil of the nucleus; and we know also that the β-particle can be detected in a scintillation counter. In the absence of a physical interaction by which the particle could be 'observed' on the way, the emitting nucleus is connected with the detector in one jump. Observing it in between creates a different phenomenon.

Motion in quantum physics is a transformation from one event to a later one. With the events, somehow or other an existence is connected. Classically, we want to put a thing at each event. The phenomenon of interference shows that we have to associate the existence with both events. The connexion between the events is an atomic existence: it cannot be cut.

The events that are connected are, in fact, not point-instants, although we may for mathematical convenience represent them as such, subject, of course, to the limited precision allowed by the Uncertainty Principle in applying the representation. So long as the events are well separated in space and time, this limitation may be left out of account in discussing the existence of atomic entities. Our idea must depend on essentially classical supports, namely, the events observed and recorded by means of devices we can see and handle, and the substantiality of the objects on which we depend to observe.

So far as physical observation is concerned where interference is involved, there is no existence between the source event and the detection event. So the kinematic order of ordinary physical space-time does not correspond to spatial possibility for the individual atomic existence. Yet the existences connect pairs of events in ordinary physical space-time. When under the stimulus of the bullet analogy for radiation we think of things launched into space, we fail to appreciate that we are ordering independent possible atomic existences into a series with a common initiating event (or one fictitiously arranged to be so) and a variable terminating one. But to get to a particular termination, one does not go through intervening members of the series, unless there are physical effects actually occurring by which to label the track. This then bears a resemblance to classical observation of a moving object.

A particle of a radiation field connects an emission event with a detection event (and it may also involve other events). The existence at the emission event is the recoil, or some such change in the emitter that can be observed. A corresponding existence is established at the detector. These two events, as it were, bound the existence of a particle which is said to leave the source and enter the detector, and is thought of as moving in the intervening space. Provided that the representation is crude enough, this view is practically effective. If sharper representation is attempted, the classical view of motion and the attendant possibilities of interpolating observations that do not disturb the physical process of the motion being observed are no longer allowed. Only if we accept the transition between the two events at the source and detector as the existence of the particle concerned are we on safe ground in relation to the interference of probability. In so far as we do not need to bring into account the physical effect of distant objects, we have the entity localized in an extended region that includes both events—but then we cannot imagine it moving within the region in a classically acceptable sense. Only if we can interpose events at which physical interactions take place that can be observed is the picture of classical motion applicable. Thus motion in quantum mechanics with non-classical statistics is represented by a set of separate existences which overlap on our map, whereas what corresponds to the classical case is a series of consecutive jumps in a continuing existence. The distinction is shown in Fig. 1.

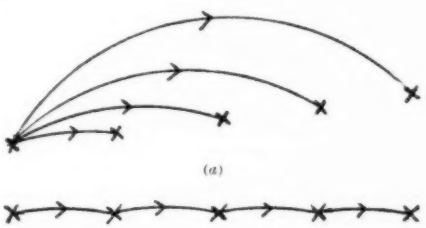

Fig. 1. The events being connected are marked ×. (*a*) Quantum connexions and overlapping existences; (*b*) classical series of consecutive jumps in a continuing existence

If we wish to represent the variable termination as a moving point governed by a continuous law of motion, we cannot have the point move in ordinary physical space because we must eliminate the intervening possibilities of ordinary physical space between source and detector.

We may exhibit in an elementary way how to achieve this. Consider the intersection of a rectangular axis triad ($OABC$) with a sphere of unit radius centred at O. Let the points of intersection be a, a', b, b', and c, c'. The directions Oa, Oa', Ob, Ob', Oc, Oc' stand for six distinct logical possibilities with which we are all familiar in ordinary language, namely, up-down, right-left, fore and aft. Vectors joining O to points (x) of the sphere other than a, a', b, b', c, c', do not stand for logical possibilities in this system. The vector Ox is a linear combination of the basis vectors Oa, Ob, Oc. (Here $Oa + Oa' = 0$, where + stands for the logical 'or'. So $Oa' = -Oa$. This is a necessary part of the logic of the system, since Oa and Oa' are mutually exclusive.) Ox does not represent one of the six logical possibilities, but it could represent a statistical combination of these possibilities, provided that we have regard to the laws of probability in establishing the representation. The mathematical elaboration of this is obvious, since the sum of the squares of the components of Ox is unity. The important point for my purpose here is that some points x are traversed by the representative point on the sphere in continuous transitions between pairs of the six logical elements of our system. A jump in the discrete system is then regarded as a continuous change according to some law which we have not yet specified. Once the continuous law is given, however, a track is specified on the sphere, and in going from Oa to Ob, for example,

would pass continuously through points between *Oa* and *Ob* on the track.

This scheme of representation may be extended to a system of n possibilities and a representation in n-dimensional linear vector space. Imagine possibilities for existence on a lattice (h,k,l) where h, k and l are integers. We shall denote a variable point of the lattice by the integer triad (ξ, η, ζ). The mathematical identity:

$$u(\xi,\eta,\zeta) = \sum_{h,k,l} u(h,k,l)\ \delta_{\xi h}\delta_{\eta k}\delta_{\zeta l}$$

$0 \leqslant h,k,l \leqslant N$, where δ_{mn} is the Kronecker delta symbol, can be read as a vector equation in the space in which each

$$e_{hkl} = \delta_{\xi h}\delta_{\eta k}\delta_{\zeta l}$$

is a basis unit vector with $e_{hkl}\,e_{h'k'l'} = 0$ or 1 according as the corresponding primed and unprimed subscripts differ or agree. $u(h,k,l)$ is the hkl-component of the vector $u(\xi,\eta,\zeta)$; it may be a continuously variable number of absolute magnitude $\leqslant 1$, and indeed, corresponding to our previous spherical representation:

$$|u(\xi,\eta,\zeta)|^2 = \sum_{h,k,l} |u(h,k,l)|^2 = 1$$

Thus we have a N^3-dimensional linear vector space in which $u(\xi,\eta,\zeta)$ is represented as a vector.

Now consider displacement from (0,0,0) to (a,b,c) on the lattice, that is, through points (ξ,η,ζ) for which the succession of co-ordinates is governed by the following rule (A); in each step only one of the integers, ξ,η,ζ, changes by unity while the other two remain unchanged. On the lattice, this resembles continuous motion in the continuum.

The corresponding transformation from $u(0,0,0)$ to $u(a,b,c)$ in the vector space proceeds via rotation from one co-ordinate axis to the next. But clearly, if u_{hkl} and $u_{h+1,kl}$ are positive:

$$u(\xi,\eta,\zeta) = u(h,k,l)\delta_{\xi h}\delta_{\eta k}\delta_{\zeta l} + u(h+1,k,l)\delta_{\xi h+1}\delta_{\eta k}\delta_{\zeta l}$$

represents a vector between e_{hkl} and $e_{h+1,kl}$. That is, whereas on the lattice we are concerned with integral steps between points of the lattice, in the vector space we can prescribe a continuous transition through the corresponding basis vectors in turn. Further, if we wish to allow only the integral connexion $(0,0,0) \rightarrow (a,b,c)$ on the lattice, we may prescribe a continuous one-parameter family of vectors bounded by $u(0,0,0)$ and $u(a,b,c)$, which includes none of the basis vectors except e_{000} and e_{abc}.

Thus this representation makes possible continuous transition (or motion) in the vector space corresponding to the connexion between two widely separated points of the lattice without requiring that intervening points of the lattice be traversed in accordance with the rule A.

Let us now pass to the representation of possibilities for existence in the continuum of physical space by letting N tend to infinity. The basis vectors now become:

$$\delta(\xi - x)\delta(\eta - y)\delta(\zeta - z)$$

corresponding to the spatial positions (x, y, z) and the sum over h, k, l is replaced by an integral over physical space. The integral connexion between two points of physical space, one corresponding to the source and the other to the detector of a physical particle, is represented in the vector space (which is now ∞-dimensional) by a continuous transition which need not require the vector to pass through any of the basis vectors.

In quantum mechanics the domain of numbers in which the components of the vectors vary is not the continuum of real numbers hitherto assumed above, but that of complex numbers (the grounds for this are given by H. Weyl in *Gruppentheorie und Quantenmechanik*, §4).

As an example of the law of continuous (unitary) transformation in the Hilbert space we may show the equation:

$$\frac{\hbar}{i}\frac{d\mathbf{x}}{dt} + H\mathbf{x} = 0$$

where $\mathbf{x}$ is the unitary vector and H is the Hermitian matrix for the energy (Weyl. *op. cit.*, §15).

In representing the emission of an atomic particle from a source, we should normally choose as the initial vector, consistent with our knowledge of the emitter, one of the characteristic outgoing spherical waves (an eigen function of the angular momentum). The location of the source is then indefinite to the extent determined by a momentum uncertainty equal to twice the momentum of the emitted particle. But when the source is observed to recoil, we have to choose a different vector to represent the outgoing particle: because of our enforced ignorance in the circumstances we have imagined, it cannot be an eigen vector of position or momentum, and it cannot be one of angular momentum. The particular vector judged appropriate for this representation carries the information needed with the dynamical law to determine the probability of the free particle being detected by a suitable detector placed in space to receive it. Thus the unitary vector associates a probability of detection with each point of physical space representing the position of a possible detector, and consequently with each member of the family of atomic existences that can be associated with the event in which the source recoils in a particular direction and 'a particle is emitted'.

If some diffracting or other physical system is interposed between source and detector, the 'state vector' for the particle is transformed by dynamical interaction and the new probability for detection at a given place is determined by the modulus squared of the component of the transformed vector in the functional direction. $\delta(x - x_D)\delta(y - y_D)\delta(z - z_D)$, (x_D,y_D,z_D) being the position of the detector.

In the functional space each possibility of existence is represented by a ray of 'unit length' from the origin. Only the particular axes that are eigen functions of 'the positional co-ordinates of the particle' correspond to the existences in physical space. Any other ray does not correspond to a point of physical space at all, because it involves in a sense every point of physical space. Thus in the functional space we have possibilities for existence that are not possibilities for existence in ordinary space. They can be interpreted statistically, however, and this is what quantum mechanics does.

In classical representation the physical thing is located in the event. This act in the representing process may stand for a quite different and more elaborate pattern of behaviour than merely marking the map, for it involves actual things and other processes. Whatever is done must be acceptable as a support for attaching to the event a name by which an existence inheres in the event. According to the conception presented here for atomic existence, we should attach the name to the atomic transition between source event and detection event. In a

crude representation in which these events are not distinguishable, the name is in effect attached to a single event, as required by the classical representation with which we use the word 'existence' in ordinary language.

As to the existence of electrons in an atom or nucleons in a nucleus, physicists seem to be in no difficulty at the level of ordinary language, because the physical forces localize the particles sufficiently well to permit its unrefined use.

TIME-INTEGRATED MEASUREMENTS OF SUBMARINE IRRADIANCE

By RONALD CURRIE and LAURENCE DRAPER
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IN the course of trials of a new type of self-recording integrating irradiance meter for use under water, some measurements of ecological interest have been made in two Scottish lochs.

The irradiance meters are designed to be suspended at a number of depths from a free-floating buoy and, in the course of a day, to integrate the total irradiance at these points. The final instruments will be designed to record the scalar irradiance in conjunction with measurements of the photosynthesis of the phytoplankton[1], but at present they have been used with flat-plate (cosine) collectors primarily to test the recording mechanism. No allowance has been made for this in presenting the results, and the integrated measurements do not take into consideration the alteration in angular distribution of the radiation as the day proceeds. Nevertheless, since the measurements were made under water, discrepancies from the true picture of downward irradiance are likely to be minimized through the effects of refraction at the surface and scattering by suspended particles in the water which tend to orientate the under-water radiation towards the vertical[2].

Through the courtesy and help of the director and staff of the Scottish Marine Biological Association Laboratory at Millport, we have been able to use these meters at two stations, one in Loch Striven and one in Loch Fyne. On both occasions the instruments were suspended at depths of 3, 5, 8·5 and 11 metres from sunrise to sunset in an unshaded position.

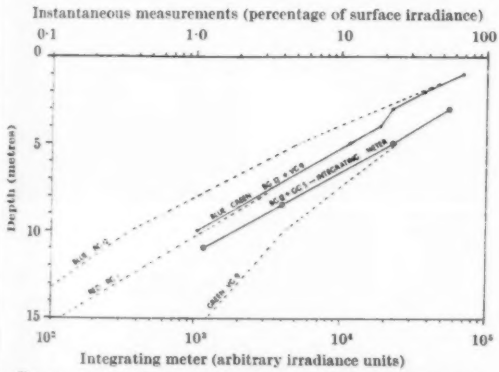

Fig. 1. Instantaneous sub-surface irradiance measurements expressed as a percentage of surface values at midday on May 17, 1961, at a station in Loch Striven. Curves are shown for the attenuation of different colours indicated by the number of the respective Schott filters used, *BG*12 giving a transmission from 360 to 480 mμ, *VG*9 from 480 to 600 mμ and *RG*1 from 600 to 720 mμ. The instantaneous measurements in the blue-green (*BG*12 + *VG*9) are compared with the integrated measurements (*BG*12 + *GG*5), the latter being expressed in arbitrary units

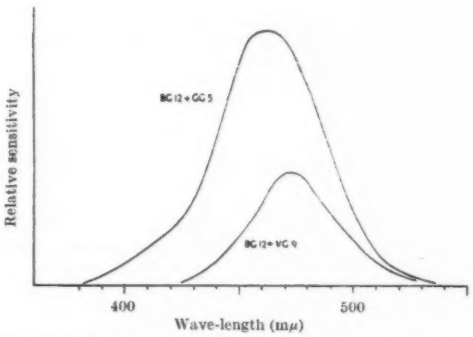

Fig. 2. The relative response of the cells and filter combinations used for blue-green instantaneous measurements (*BG*12 + *VG*9) and integrated measurements (*BG*12 + *GG*5)

Typical integrated measurements are shown in Fig. 1, where they are compared with instantaneous measurements which were made at midday on both occasions by the method recommended by Atkins *et al.*[3].

The integrating meters are fitted with a filter combination consisting of a 1-mm. thickness each of Schott *BG*12 and *GG*5 glass. The spectral sensitivity of these filters in conjunction with the photocell (Fig. 2) covers a 50-mμ band centred on the wavelength of maximum transmission in oceanic waters. This sensitivity lies close to that of the meters used for instantaneous attenuation measurements in the blue-green (Fig. 2) which were fitted with a combination of 2 mm. thicknesses of Schott *VG*9 and *BG*12 glasses.

Comparing the measurements in this part of the spectrum, it is clear that the decrease with depth of the irradiance integrated over a whole day follows a pattern very close to that of the attenuation measured instantaneously at midday. The corresponding attenuation coefficients 2·30 ($\log I_{\lambda,z} - \log I_{\lambda,(z+1)}$) (ref. 4) are shown in Table 1.

The close similarity between these measurements is very interesting, for it lends support to a method

Table 1. Attenuation Coefficients for Instantaneous Measurements and for the Irradiance over the Duration of a Daylight Day, in the 5–10 m. Layer in Two Scottish Lochs

	Date	Instantaneous measurement Filters *BG*12 + *VG*9	Irradiance integrated over one day Filters *BG*12 + *GG*5
Loch Striven	May 17, 1961	0·482	0·493
Loch Fyne	May 18, 1961	0·532	0·559

commonly used in marine ecology in which the total radiant energy at different depths in the sea is computed by applying instantaneous attenuation measurements to an integrated record of the sea surface irradiance[5,6].

To the best of our knowledge these are the first direct measurements of sub-surface irradiance measured over a protracted period. While the agreement between these and the instantaneous measurements is good, it should be remembered that only the downward sub-surface irradiance has been considered, and that the measurements were made under ideal conditions with only slight sea-surface disturbance and a relatively high noon solar altitude (53°). We hope to extend these measurements to a consideration of scalar irradiation under water in diverse weather conditions.

Finally, an interesting feature of the optical characteristics of the water in these two lochs is demonstrated by the very high rate of attenuation of blue light (Fig. 1) measured with a Schott *BG*12 filter with its maximum transmission at about 430 mμ. This strongly selective absorption must only be ascribed to a strong yellowish coloration of the water, probably from dissolved organic substances like the "gelbstoff" of Kalle[7] and perhaps for the greater part originating from the humic acids in the fresh water inflow into these lochs.

A description of the integrating irradiance meters will be published shortly[8].

[1] Currie, R. I., *U.G.G.I. Symp. Radiant Energy in the Sea*, Helsinki, 1960 (in the press).
[2] Tyler, J. E., *Bull. Scripps Inst. Oceanogr.*, **7**, No. 5, 363 (1960).
[3] Atkins, Clarke, Petterson, Poole, Utterback and Ångström, *J. du Conseil*, **13**, 1, 37 (1938).
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[6] Talling, J. F., *New Phyt.*, **56**, 29 (1957).
[7] Kalle, K., *Conseil. Perm. Int. Explor. Mer, Rapp. et Proc-Verb.*, **109**, Pt. 3 (1939).
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FIBROUS SILICA

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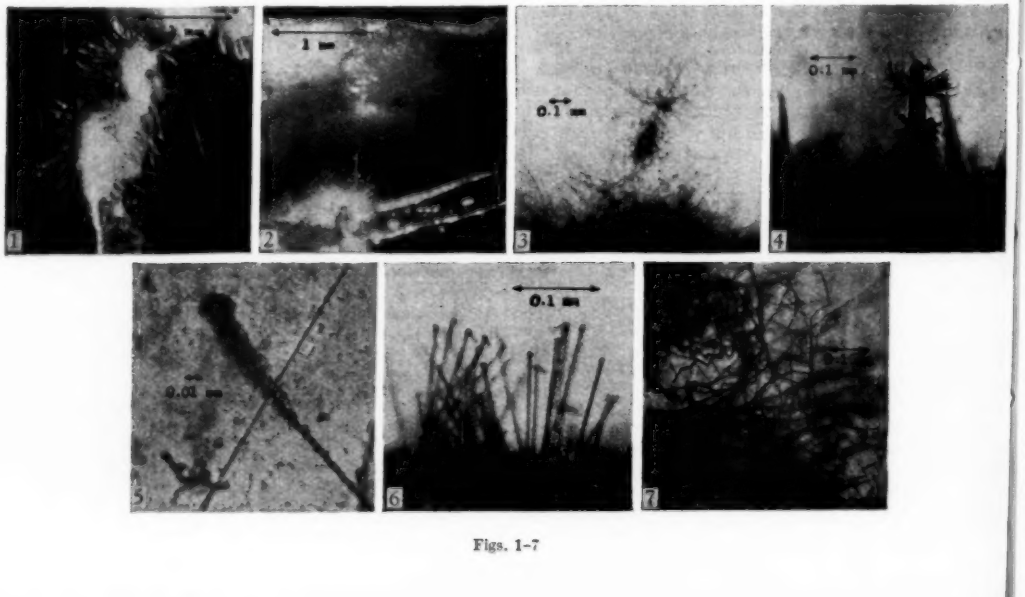
FIBRES of silica were grown on electrically heated platinum surfaces by exposing the platinum target to nitrogen-diluted mixtures of silicon tetrafluoride and water vapour. Before striking the target, the mixture was passed through an electrically heated platinum tube maintained above the temperature of the target. The growth of the fibres on the target could be observed microscopically.

The fibres so obtained have circular cross-sections without any visible indication of faces. Diameters of fibres range from less than 1μ to 50μ. Their lengths range from 10μ to millimetres, and are limited mainly by the availability of growth space which has a homogeneous temperature field.

The refractive index of the fibres approximates the known refractive indices of vitreous silica (1·46) and is lower than the indices of any known forms of crystalline silica. Rotation of a fibre around its longitudinal axis reveals no optical anisotropy. Birefringency can be observed by rotating the fibres around axes perpendicular to their longitudinal axis. Because of the curvature of the fibres and the rather slight birefringency, it is difficult to distinguish if the birefringence extends uniformly over the full length or is segmentally modulated.

Figs. 1–7 illustrate typical forms of fibres. All fibres were grown at target temperatures of approximately 1,100° C. Lowering the target temperature



Figs. 1–7

produces colloidal silicious deposits, rather than fibres. Fibres start to grow straight or slightly curved, mostly originating from limited areas of the target. Clusters of this kind are shown in Figs. 1 and 2. The cluster in Fig. 1 originates from a corner of the platinum target. The cluster in Fig. 2 grew in a fan-shaped arrangement around the circumference of a thermocouple wire.

Frequently, secondary branching can be observed, as shown in Figs. 3 and 4. In these pictures the parental fibres are still visible. Another secondary growth occurs if fibres grow out of the optimal-temperatures field or if temperature fluctuations occur. In this case, the fibres terminate their growth with heads of opaque finely dispersed silica (Fig. 1) or the surface of the fibre becomes coated with colloidal silica (plume-shaped growth at the centre of Fig. 5).

Observation of fibre growth under the microscope conveys the impression that the fibres are in a plastic state. A fibre, having terminated its growth, may become shorter and thicker (Fig. 4) or may develop a spherical head at the point (Fig. 6). These spherical heads are as transparent as the rest of the fibre, while the colloidal deposits are opaque to light. The thickened fibre in Fig. 4 added later a mustachioed growth to its obese shape. Occasionally fibres suddenly change their direction of growth; the helical and zigzag shaped fibres shown in Fig. 7 owe their form to spontaneous angular bending of already grown, originally straight fibre segments. In addition to this drastic bending at distinct regions of the fibres, which is not reversible and which appears to be the product of flow or slip, the fibres constantly perform slight twisting and bending motions of synchronous character. Microscopical observation at growth temperature gives the impression of looking on a colony of water plants, drifting in a slow current. These motions are cyclic and reversible and may well be due to strains produced by fluctuations in the temperature field.

Spectrochemical analysis of the fibres produced only the emission lines of silicon. Water and fluorine contents were not determined, but are expected to be low since these compounds and their possible reaction products with silica have high vapour pressure at growth temperatures.

X-ray diffraction investigations of a multitude of fibres by the powder method showed amorphous bands plus faint but distinct diffraction lines, indicating a small degree of crystallinity. Selected fibres were mounted and investigated by single-crystal X-ray diffraction methods, but were only found to be completely amorphous.

A more detailed study of the fibre-forming conditions and more structural studies are being pursued. The most likely cause for the deposition of the silica into oblong, statistically and energetically unfavourable forms, is the action of some directing lattice force, although other directing mechanisms such as electrostatic agglomeration and thermal effects may be possible.

PYROLYSIS AND GAS-LIQUID CHROMATOGRAPHY ON THE MICROGRAM SCALE

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WE are now able to describe an improved pyrolyser unit for use in conjunction with a gas-liquid chromatograph.

Whereas the previous model[1], designed for use with a chromatograph equipped with a gas density balance detector[2], required a sample size of 2–3 mgm., the new unit, coupled with a Pye argon chromatograph, needs only 20–30 μgm. In this way the essential requirements for pyrolysis are better observed, in particular the criterion of a very thin sample film.

The improved pyrolyser (Fig. 1) consists of a 22-cm. length of 1 × 0·0015 single core 'Pyrotenax' so fashioned at the lower end that the sheath is partially cut away for 5 cm. and the insulating medium removed; the central conductor is shortened by 4 cm. and the end turned over to form a hook. A brass bush, 6 mm. long and 2·5 mm. in diameter, drilled to 20 wire gauge along its major axis and drilled and tapped to accept a 12 *BA* grub screw as illustrated, is brazed on to the inside of the cut-away at the bottom of the sheath. The filament is a 10 cm. length of 34 s.w.g. nichrome wire coiled to a diameter of 1·5 mm. (about 10 turns) one end of which is hooked over the end of the central conductor while the other is held captive in the brass bush after the length has been adjusted to give a resistance of 2 ohms. The upper end of the 'Pyrotenax' has the sheath removed for about 1·5 cm. to expose the central conductor and connexions are made in a conventional manner. The glass *T*-piece consists of a *B*.14 cone and a side entry for the carrier gas stream;

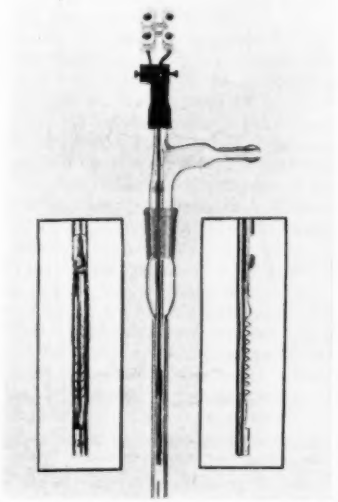

Fig. 1. General view of improved pyrolyser with details showing the filament mounting

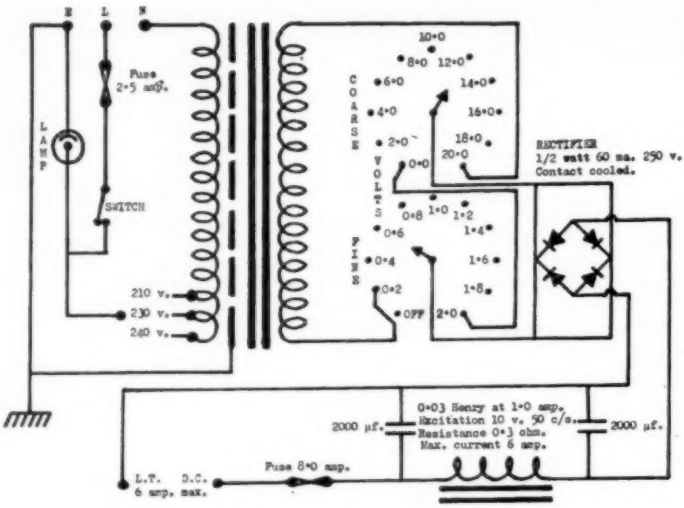

Fig. 2. Firing circuit for improved pyrolyser

the 'Pyrotenax' is held in position by means of a short length of thick-walled rubber tubing. The firing circuit (Fig. 2) is the low-tension section of a Radford 'Labpack'[3], which gives 0–22 V. by 0·2 V. steps up to a load of 6 amp.

The filament is calibrated by plotting applied potential versus temperature (measured under actual operating conditions with a Cambridge disappearing filament pyrometer).

The sample, in the form of a dilute solution or dispersion (about 1 per cent w/w concentration), is applied with a fine camel-hair brush to one or two coils toward the centre of the filament and dried under an infra-red lamp.

Comparison of the chromatograms obtained by pyrolysing a styrene homopolymer on both the milligram and the microgram scales (Fig. 3) is of interest, for the monomer yield rises from about 90 per cent on the milligram scale to near quantitative on the microgram scale. This, and the analogous behaviour of a methyl methacrylate homopolymer, are evidence for the occurrence of secondary reactions in the thicker film, probably due to the diffusion of fragments through the degrading polymer despite a reasonably short time of residence in the vicinity of the filament[4]. It is clearly advantageous, therefore, to work at the microgram-level.

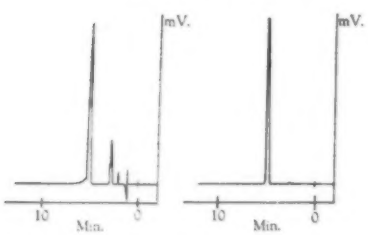

Fig. 3. Comparison of the pyrolysis chromatograms of a styrene homopolymer on the milligram scale (left) and the microgram scale (right) under otherwise similar conditions

The catalytic effect of the filament surface can be demonstrated at the milligram-level by comparing the pyrolyses of styrene and methyl methacrylate homopolymers using nichrome and gold-plated nichrome wires; the latter surface gives slightly simpler chromatograms. However, at the microgram-level no such differences are apparent when the pyrolyses are conducted on nichrome, gold-plated platinum and platinum filaments.

The versatility of the technique is illustrated by the following examples:

(i) Although many synthetic polymers are readily soluble in a wide range of organic solvents, it is often desirable to sample an aqueous polymer emulsion or a modified aqueous polymer emulsion direct without

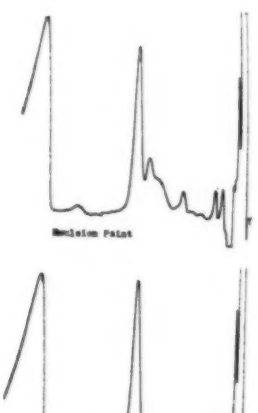

Fig. 4. Pyrolysis chromatograms of an emulsion paint and the emulsion base obtained by pyrolysing at 700° C. and chromatographing at 82° C. on a 4-ft. column prepared with 12½ per cent w/w poly-(ethylene glycol succinate) on 100–120 mesh acid-washed 'Celite' 545. Detector setting, 1,250 V. at maximum sensitivity. Argon carrier gas at 24 ml./min. Chart speed, 6 in /hr. Time of each analysis, 40 min.

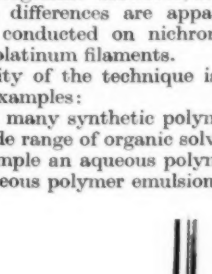

Fig. 5. Pyrolysis chromatogram of a sample of a motor-cycle fairing. The shaded peak is due to styrene. Conditions as for Fig. 4. Analysis time, 60 min.

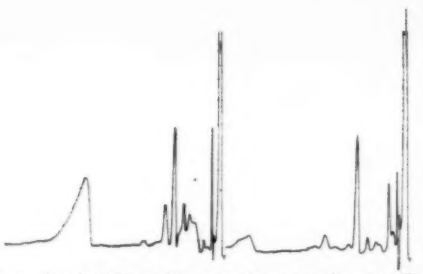

Fig. 6. Pyrolysis chromatograms of two samples of a silicone rubber. Sample 1 (left), sample 2 (right). Conditions as for Fig. 4. Time of each analysis, 50 min.

any preliminaries. This is exemplified in Fig. 4, which shows the characteristic identity of the polymer without separation from the inert constitutents.

(ii) Fig. 5 shows application to a fabricated polymer, a glass-fibre reinforced polyester, sampled by filing with a smooth Swiss file; the resultant powder mixed with 1 per cent w/w of gum tragacanth is slurried with water and then applied to the filament. Obviously, many materials may be treated in this way if they cannot be taken up into solution; a sample such as coal, for example, may be ground to an impalpable powder. It is desirable that filed or powdered samples should pass a 200 B.S.S. sieve.

(iii) Fig. 6 illustrates the pyrolyses of two specimens of a high-temperature silicone rubber, sampled by filing at − 70° C. (grinding at a somewhat lower temperature would be equally effective). Silicone rubber No. 1 retains its properties after prolonged use at 300° C., while silicone rubber No. 2 breaks down in use at 200° C. Quite clearly, there are chemical differences between the specimens.

We wish to thank the Directors of Vinyl Products, Ltd., for permission to publish this article, and we are indebted to Mr. G. E. J. Reynolds for helpful advice, and to Mr. M. Harrison for assistance with the practical work. Thanks are also due to Mr. F. Jackson, of the Woodall-Duckham Construction Co., Ltd., for the loan of a disappearing filament pyrometer.

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EFFECT OF POLYVINYL ALCOHOL ON THE DISTRIBUTION OF IODINE-131 IN THE INTERNAL ORGANS, FLUIDS AND EXCRETA OF RABBITS

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ON the basis of experiments on mice given aqueous solutions of polyvinyl alcohol to drink in concentrations of 0·1 per cent–10 per cent for 210 days, it was found that this polymer is a non-toxic compound and causes a slight increase in the glycogen in the liver[1]. Experiments carried out with polyvinyl alcohol on rabbits showed that an 8 per cent solution at *p*H 7·3 and viscosity 1·7 possesses properties similar to plasma. This solution, when injected intravenously, brought about an excessive deposition of glycogen in the liver cells and Browicz-Kupfer cells during the first 4 weeks after injection, and, 3 months after injection, the liver glycogen content was similar to that of the controls. An increase in the number of normoblastic cells was noted in the marrow[2]. In experiments on *Tendipes* f. l. *plumosus* in aqueous solutions of polyvinyl alcohol, a 50 per cent increase in hæm was discovered[3]. Polyvinyl alcohol, administered intravenously to rabbits together with a shock dose of trypan blue, was effective in preventing the appearance of shock symptoms, and when administered to rabbits during the initial phase of shock, diminished the shock symptoms and thus prevented the death of the animals[4]. This polymer can, in addition to the above-mentioned properties, bind dyes and toxins and increase the kidney excretion coefficient of these substances.

In view of these properties, revealed in our previous experiments, our aim was to examine the effect of polyvinyl alcohol on the distribution of iodine-131 in animal organs.

The experiments were carried out on 20 rabbits of both sexes, weighing between 2·5 and 3 kgm. The rabbits were divided into two series, and each series was divided into two groups, (*a*) and (*b*). Rabbits 1–5, group (*a*), series I, were given 10-μc. iodine-131 intravenously in 10 ml. of physiological salt solution. Rabbits 6–10, group (*b*), series I, received 10 μc. iodine-131 in 10 ml. of polyvinyl alcohol, intravenously. All the rabbits of series I were killed 4 hr. after administration of iodine-131 with physiological salt solution and polyvinyl alcohol.

Rabbits 11–15, group (*a*), series II, received intravenous injections of 10 μc. iodine-131 in 10 ml. of physiological salt solution. Rabbits 16–20, group (*b*), series II, were given 10 μc. iodine-131 in polyvinyl alcohol, intravenously. All the rabbits of series II were killed 17 hr. after injection. Before killing the animals of both series, 3 ml. of blood were taken for testing. After centrifuging, the serum was separated from the morphological elements. When the rabbits had been killed, they were dissected. In order to detect radioactivity, the urine, fæces, bile, parenchymal organs, endocrinal glands, lymphatic nodes and marrow were taken for testing. All the tissues were dried at 80° C., after which they were triturated in a mortar, and the resulting powder put into specially prepared bags of polyvinyl chloride (as a protection against radiation). After weighing, the powder was placed under the mica end-window of the chamber in the Geiger–Müller, BAT-type counter and the number of impulses in a certain period of time was recorded.

At the same time, sections of organs were taken from each rabbit for histological examination. They were fixed in 6 per cent formalin solution and then,

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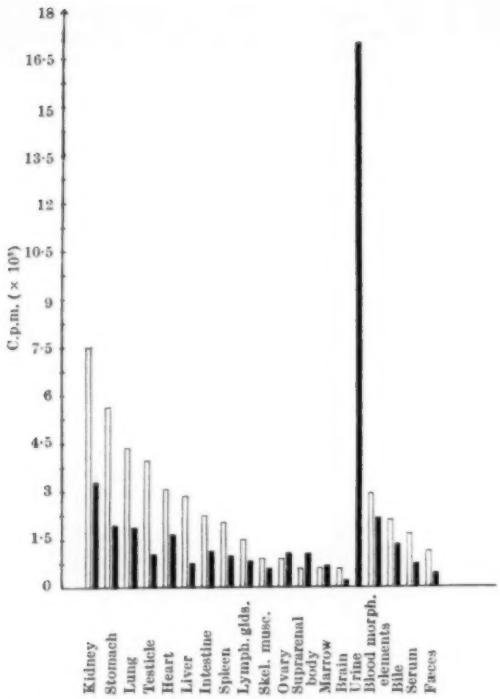

Fig. 1. Average findings from 5 rabbits injected intravenously with iodine-131 in physiological salt solution and from 5 rabbits given labelled iodine in 8 per cent aqueous solution of polyvinyl alcohol. The rabbits were killed 4 hr. after injection. The various columns represent activity of 1 gm. of dry mass of the organs of fluids, and of excreta, expressed in impulses per min. after intravenous injection of: white, iodine-131 in physiological salt; black, iodine-131 in polyvinyl alcohol

after dehydration, embedded in paraffin. The sections were stained with hæmatoxylin and eosin. The results are given in Figs. 1–3. The various columns represent 1 gm. of dry organic mass, fluids and excreta in counts per min. after intravenous injection of iodine-131 in physiological salt or polyvinyl alcohol. In calculating the radioactivity, self-absorption of radiation in the various samples was taken into consideration.

In the microscopical picture of the lungs of series I, group (*a*) rabbits, the walls of the alveoli were thickened. The epithelial cells of the alveoli were œdematous, with strongly acidophilic protoplasm. The mucous membrane of the bronchus showed hypertrophic and hyperplasic traits. The same changes were evident in the capillary vessels of the endothelium and the parabronchiole branch of the pulmonary artery. In the walls of the lung alveoli and in the blood vessel lumen, a great number of acidophilic leucocytes were seen. In the parabronchiole lymphatic nodules, hypertrophic and hyperplastic characteristics were observed.

The cardiac muscle displayed basophilic degeneration as well as single acidophilic leucocytes. The liver cells were enlarged, their trabecular outline obliterated and the nuclei of the Browicz–Kupfer cells clearly hyperchromatic. In the portal spaces, single acidophilic leucocytes were evident.

The bile duct epithelium showed hypertrophic characteristics. The epithelial cells of the straight kidney caniculi had a light-coloured protoplasm and gave the impression of being swollen. Among them, single acidophilic leucocytes were observed.

The suprarenal body displayed hypertrophy of the cortical layer with focal hyperplasia of the glomular layer. Single acidophilic leucocytes were seen in them. The stomach mucous membrane contained, in the superficial layer, single acidophilic cells. The lymphatic nodules of the appendix possessed hypertrophic characteristics and a considerable admixture of acidophilic cells. The glandular epithelium of the mucous membrane was grossly swollen and in places mucously degenerated.

In the rabbits of series II (*a*), killed 17 hr. after injection, there were considerably less acidophilic cells; on the other hand, mononuclear cells, similar in appearance to plasmatic cells, were increased in number.

In the sections of rabbit organs from series I and II (*b*) with polyvinyl alcohol, the acidophilic cells were less numerous than in group (*a*), with the physiological salt solution. The changes in the cells were absent, nor was there evidence of hyperplasia.

It was experimentally demonstrated that the rabbits which were given iodine-131 with polyvinyl alcohol were found to have a considerably different content of iodine-131 (in counts per min.) in their various organs, fluids and excreta, as compared with the rabbits given iodine-131 in physiological salt solution. In the thyroid gland, 4 hr. after administration of iodine-131 with polyvinyl alcohol, the radioactive iodine which accumulated was six times greater than in the thyroid of rabbits given iodine-131

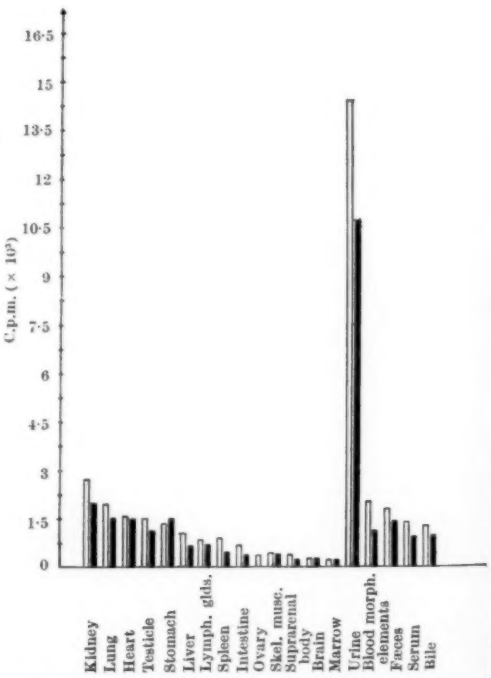

Fig. 2. Average findings from 5 rabbits injected intravenously with iodine-131 in physiological salt solution and from 5 rabbits injected intravenously with iodine-131 in an 8 per cent aqueous solution of polyvinyl alcohol. The rabbits were killed 17 hr. after injection. The various columns represent the activity of 1 gm. of dry mass of the organs, fluids or excreta expressed in impulses per min. after intravenous injection of: white, iodine-131 in physiological salt; black, iodine-131 in polyvinyl alcohol

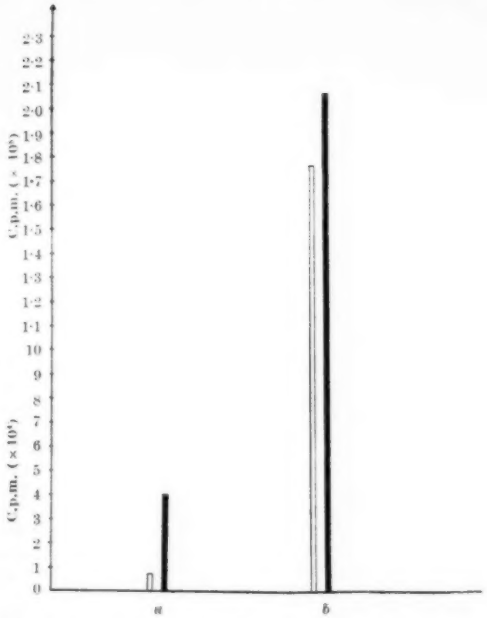

Fig. 3. Average findings from 5 rabbits which were given intravenous injections of iodine-131 in physiological salt solution and from 5 rabbits injected with iodine-131 in 8 per cent aqueous solution of polyvinyl alcohol. The rabbits were killed (*a*) 4 hr. after, (*b*) 17 hr. after injection. The various columns represent the activity of 1 gm. dry mass of degenerated thyroid, expressed in counts per min. after intravenous administration of: white, iodine-131 in physiological salt; black, iodine-131 in polyvinyl alcohol

with physiological salt solution. However, in the parenchymal organs of these rabbits, the amount of iodine-131 was less (by practically half) than that of the iodine-131 in the organs of the rabbits to which it was administered in physiological salt solution. After the injection of polyvinyl alcohol, the rabbits excreted urine in large amounts, especially during the first 4 hr. We recorded 1,650 counts per min. in the urine. Anuria was observed to occur during the first 4 hr. in the rabbits given iodine-131 with physiological salt solution. 17 hr. after injection of iodine-131 with polyvinyl alcohol, there was still more radioactivity to be found in the thyroid of these rabbits than in those which had received iodine-131 with physiological salt solution. Radioactive iodine accumulating in the parenchymal organs had a harmful effect on these organs, as was seen from the microscope pictures. Administration of iodine-131 with polyvinyl alcohol caused it to collect more rapidly in the parenchymal organs and thus the possibility of injury to these organs is considerably reduced.

In our experiments on rabbits, we also showed that the radioactive iodine given with polyvinyl alcohol and collected in the thyroid gland was six times greater than when given in physiological salt solution. Therefore, it can be concluded that doses of iodine may be reduced to a sixth, and thus the pathological changes in the parenchymal organs, occurring on administration of radioactive iodine, are even further diminished.

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CONSTITUTION OF CELLULOSE MICROFIBRILS

By D. T. DENNIS and Prof. R. D. PRESTON, F.R.S.
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THE difficulty in isolating a native cellulose yielding glucose only on hydrolysis has long been recognized. Until comparatively recently the non-glucose sugars included in the hydrolysates of cellulose have been regarded as derived from residual unextracted hemicelluloses. The identification of cellulose with the microfibrils visible in the electron microscope, and the evidence that apparently pure microfibrillar material yield other sugars as well as glucose sometimes in amounts up to 50 per cent of the total sugar[1], show that the microfibrils are more complex than at first sight appeared. Since the microfibrils of, for example, *Rhodymenia* cellulose yield approximately equal amounts of glucose and xylose then they must at least contain molecular chains both of xylose and glucose. It is equally possible that the molecular chains may be mixed.

On various grounds it appears reasonable to suppose that the chains running down the centre of a microfibril form a core which is highly crystalline and that this core is clothed in a 'cortex' in which the chains, while still parallel, are not regularly spaced[2]. It is clearly possible to suggest further that this difference in the degree of crystallinity could be due to the presence in the cortex only of, for example, xylose residues, whether in the form of xylan chains or of mixed glucan-xylan. This possibility can to some extent be tested.

According to Rånby[3] and, later, Mukherjee and Woods[4] and others, cellulose microfibrils can under certain conditions be broken down to short rodlets which Rånby identifies with the crystallites—micelles—first suggested by Nägeli as the crystalline units in cellulose and later found necessary to explain some features of the X-ray diagram. If it were possible even approximately to associate these rodlets with short lengths of the crystalline core of the microfibril then analysis of these rodlets would serve to decide whether or not this central core is homogeneous. This article concerns the relevant observations.

Holocelluloses varying widely in hemicellulose content were prepared by the method of Jermyn and Isherwood[5]. With the exception of *Porphyra* these were treated with 4 per cent potassium hydroxide at 20° C. for 4 hr., a process known to remove most of the alkali-soluble hemicellulose with no damage to the microfibrils. Part of the residue was hydrolysed and the sugars identified as described by Cronshaw *et al.*[1]. The rest was treated with 2·5 *N* sulphuric acid at 100° C. for 24 hr. and washed until it formed a colloidal solution[3]. The dispersed phase

Table 1

Source of cellulose	Holocellulose *		Holocellulose after 4 per cent KOH *		α-Cellulose †		Colloidal cellulose *		Acid from colloidal cellulose *	
Elm wood	glucose	*S*	glucose	*S*			glucose	*S*	glucose	*S*
	xylose	*M*	xylose	*W*					xylose	*S*
Pine wood	glucose	*S*	glucose	*S*			glucose	*S*	mannose	*S*
	mannose	*M*	mannose	*M*			mannose (trace)		xylose	*S*
	xylose	*M*	xylose	*W*					glucose	*S*
									galactose	*W*
Rhodymenia	glucose	*S*	glucose	*S*	glucose	*S*	glucose	*S*	glucose	*W*
	xylose	*M*	xylose	*S*	xylose	*S*	xylose (trace)		xylose	*S*
Ulva	glucose	*S*	glucose	*S*	glucose	*S*	glucose	*S*	glucose	*S*
	xylose	*W*	xylose	*W*	xylose	*S*			xylose	*M*
	rhamnose (trace)		rhamnose (trace)						rhamnose	*W*
Laminaria	glucose	*S*	glucose	*S*	glucose	*S*	glucose	*S*	glucose	*S*
	uronic acid	*W*	uronic acid	*W*	uronic acid	*M*				

* *S*, strong; *M*, medium; *W*, weak, spot on chromatogram.
† From Cronshaw *et al.* (ref. 1).

was precipitated by the addition of a few drops of 0·1 *N* hydrochloric acid and the precipitate was washed by centrifugation into acetone and dried *in vacuo*. The resulting powder was hydrolysed and the constituent sugars determined both in this hydrolysate and in the acid extract. At each stage the material was examined by electron microscopy and by X-ray diffraction analysis.

The analysis of the celluloses after the various treatments is given in Table 1.

The colloidal celluloses contain at most only traces of non-glucose sugars even though these are abundant in the parent microfibrillar material.

Breakdown of microfibrils to rodlets therefore involves the removal of the bulk at least of non-glucose sugars. Since, as shown for phloem fibres by Rånby[3] and by Mukherjee and Woods[4] and for our material during the present investigation, the rodlets still yield the X-ray diagram of cellulose I the molecular chains cannot have been widely separated at any stage of extraction, and it seems likely that they represent in only slightly modified form the original central core of the microfibrils. The evidence suggests therefore that this crystalline core is cellulose in the 'true' sense (the 'eucellulose' of Myers and Preston[6]) and that the non-glucose sugars of the microfibrils are confined to the outer layers. This is of considerable importance both in terms of the mechanism whereby the microfibrils may be synthesized and because it means that even in celluloses containing a low percentage of non-glucose sugar residues the exclusion of these from the central core increases the proportion of them lying on the surface of the microfibrils to a level greater than might otherwise be expected. Except when so rigorously extracted that a cellulose preparation falls to a colloidal solution, its reactions with non-swelling material may be those of xylan and/or mannan and not of a glucan.

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BREAKDOWN OF [2-^{14}C]-GLYCINE DURING PAPER CHROMATOGRAPHY

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In the course of work on the metabolism of [2-^{14}C]-glycine by animal tissues, it has been observed that up to 40 per cent of the glycine may decompose with the formation of a number of radioactive substances when paper chromatograms are developed with phenol/water followed by *n*-butanol/propionic acid/water. It seems probable on the basis of chromatographic properties that the major breakdown product is neither glyoxylic nor glycollic acid. Unless quantitative measurements were made, the decomposition of glycine would not normally be detected, since the breakdown products did not give a positive ninhydrin reaction. Fowden[1] has reported losses of up to 30 per cent with glycine when chromatograms developed with phenol/water were dried with hot air. The influence on the rate of breakdown of glycine by various solvents, and of pre-treatment with oxalic acid of the paper used for chromatography, has been investigated.

[2-^{14}C]-glycine (0·1 μc.; 0·026 μmole) (Radiochemical Centre, Amersham, Bucks) was spotted on Whatman No. 4 filter paper, either untreated or previously immersed for 2 hr. in 1 per cent (w/v) oxalic acid, and then rinsed ten times during 48 hr. with distilled water. Chromatograms were run using the following solvent systems: (i) phenol (72 per cent w/w)/water (British Drug Houses phenol detached crystals B.P.C.); (ii) phenol (72 per cent w/w)/water, after distillation of the phenol over 0·1 per cent (w/w) aluminium turnings and 0·05 per cent (w/w) sodium bicarbonate[2]; (iii) *n*-butanol/propionic acid/water (45·9, 23·4, 30·6 per cent v/v)[3]; (iv) pyridine (80 per cent v/v)/water. One-dimensional chromatograms were run using each solvent on both oxalic acid-treated and untreated paper. Two-dimensional chromatograms were run on both types of paper using phenol/water or redistilled phenol/water followed by *n*-butanol/propionic acid/water, and again with the

Table 1. EXTENT OF GLYCINE BREAKDOWN DURING CHROMATOGRAPHY WITH VARIOUS SOLVENTS, AND THE EFFECT OF PRETREATING THE PAPER WITH OXALIC ACID

Pretreatment of paper	Dimensions	First solvent	Second solvent	Carbon-14 (per cent) not in glycine after chromatography	Carbon-14 (per cent) not glycine appearing during drying of phenol*	Protective effect of using the acid solvent in the first dimension*
None	1	Ph.	—	13·5	—	—
,,	1	Redist.-Ph.	—	4·4	—	—
,,	1	But.-Prop.	—	2·1	—	—
,,	1	Pyr.	—	4·7	—	—
,,	2	Ph.	But.-Prop.	37·6	22·0	—
,,	2	Redist.-Ph.	But.-Prop.	21·8	15·3	—
,,	2	But.-Prop.	Ph.	12·5	—	− 3·1
,,	2	But.-Prop.	Redist.-Ph.	2·0	—	− 4·5
Oxalic acid	1	Ph.	—	12·8	—	—
,, ,,	1	Redist.-Ph.	—	1·1	—	—
,, ,,	1	But.-Prop.	—	0·2	—	—
,, ,,	1	Pyr.	—	2·4	—	—
,, ,,	2	Ph.	But.-Prop.	22·6	9·6	—
,, ,,	2	Redist.-Ph.	But.-Prop.	4·1	2·8	—
,, ,,	2	But.-Prop.	Ph.	8·6	—	− 4·4
,, ,,	2	But.-Prop.	Redist.-Ph.	0·9	—	− 0·4

* Calculated as: sum of breakdown in both solvents − (breakdown in phenol/water + breakdown in *n*-butanol/propionic acid/water).
Abbreviations: Ph., phenol/water; Redist.-Ph., redistilled phenol/water; But.-Prop., *n*-butanol/propionic acid/water; Pyr., pyridine-water.

solvents used in the reverse order. All chromatograms were dried for 24 hr. in a current of air at room temperature. Radioactive substances on the chromatograms were located by radioautography using 'Kodirex' No-Screen X-ray film (Kodak, Ltd.), and the radiocarbon in each compound was counted directly on the paper with an end-window Geiger–Müller tube[4] connected to a scaler.

The results are presented in Table 1, which reports the percentage of the carbon-14 on each developed chromatogram present in substances other than glycine. With phenol as the first solvent, there was streaking of radioactive material ahead of the glycine spot, suggesting breakdown of the glycine during chromatography. When this was followed by development in the second dimension with *n*-butanol/propionic acid/water, the streaked material was well separated from glycine (Fig. 1). In the acid solvent, the most radioactive area of the streaked material lay directly ahead of glycine, and it is probable that this represented glycine which decomposed while the chromatogram was being dried following development with phenol. Development in the acid solvent then separated this material from glycine. From the sum of the extent of glycine breakdown during development in phenol/water and in *n*-butanol/propionic acid/water, estimates were made of the amount of decomposition of glycine while phenol was being removed from the paper by aeration: these values are included in Table 1.

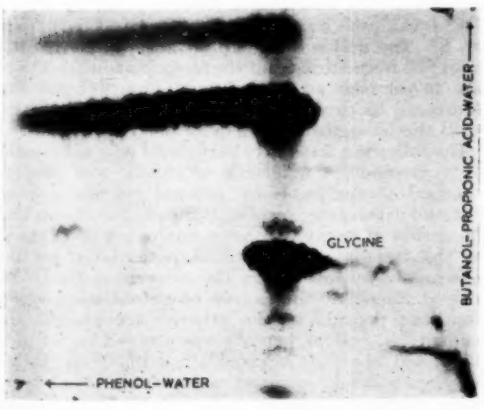

Fig. 1. Radioautogram of chromatogram of [2-^{14}C]glycine. Paper: Whatman No. 4 pretreated with oxalic acid. Solvents: phenol/water (first dimension); *n*-butanol/propionic acid/water (second dimension)

The breakdown of glycine during chromatography in *n*-butanol/propionic acid/water and in pyridine/water was low with untreated paper, and was reduced by treating the paper with oxalic acid. Breakdown in phenol/water was largely eliminated by distillation of the phenol, and there was a further improvement on treatment of the paper with oxalic acid, particularly with respect to the amount of breakdown of glycine during drying of the chromatogram. Development of the chromatogram with *n*-butanol/propionic acid/water prior to phenol/water exerted a protective effect on the decomposition of glycine in phenol, since the total breakdown after consecutive development in these two solvents was less than the sum of the observed breakdowns in each solvent separately.

Dr. J. R. Catch, Radiochemical Centre, Amersham, Bucks, has suggested that the extent of breakdown of glycine might be related to the very small quantities (2 μgm.) of the amino-acid which were applied to the paper. Three chromatograms were run using untreated paper, with phenol/water as the first solvent, and *n*-butanol/propionic acid/water as the second. [2-^{14}C]Glycine (0·1 μc.; 2 μgm.) was applied to the paper, either with no carrier or in the presence of 20 μgm. or 200 μgm. of unlabelled glycine. The percentages of the total carbon-14 not in glycine after development of the chromatograms were as follows: no carrier, 46·3; 20 μgm. of carrier, 39·4; 200 μgm. of carrier, 30·5. Thus, the breakdown of glycine was influenced by the total quantity of the amino-acid present on the chromatogram, but even large amounts of carrier provided partial protection only.

Fleming and Crosbie[5] have reported recently a non-enzymic transamination between glycine and glyoxylate which is catalysed by hydroxyl ions and inhibited at *p*H values less than 6. At *p*H 7 the reaction was catalysed by copper ions and inhibited by ethylenediamine tetraacetic acid. The *p*H of our paper, with and without treatment with oxalic acid, and before and after running in various solvents, is given in Table 2. Semi-quantitative determinations of the copper content of the solvents were performed by the phosphomolybdic acid method described by Feigl[6], and these values are reported in Table 3. It appears

Table 2. Comparison of *p*H of Oxalic Acid-treated and Untreated Paper before and after Development in Each Solvent (Abbreviations as for Table 1)

Pretreatment of paper	*p*H of paper before development	Solvent	*p*H of paper after development
None	6·7	Ph.	5·5
..	6·7	Redist.-Ph.	5·5
..	6·7	But.-Prop.	4·8
..	6·7	Pyr.	6·8
Oxalic acid	4·9	Ph.	4·8
.. ..	4·9	Redist.-Ph.	4·8
.. ..	4·9	But.-Prop.	3·3
.. ..	4·9	Pyr.	5·0

Table 3. Copper Content and *p*H of Solvents (Abbreviations as in Table 1)

Solvent	*p*H	Approx. copper content (μgm./ml.)
Ph.	4·8	20
Redist.-Ph.	4·8	20
But.-Prop.	2·5	10
Pyr.	9·2	0

from these results that the copper content of the solvents is not an important factor affecting glycine breakdown during chromatography, since there was no detectable difference in the amounts of copper present in the phenol before and after distillation; yet the extent of glycine breakdown was greatly reduced by distillation. The *p*H of the paper and of the solvents would seem to be of greater significance, as the degree of glycine breakdown on oxalic acid-treated paper (*p*H 4·9) was always less than with untreated paper (*p*H 6·7), even when pyridine/water (*p*H 9·2) was used as the solvent. The protective effect on glycine of developing the chromatogram first in the acid solvent is probably due to the incomplete removal of the acid during drying in air, resulting in a low *p*H of the paper after development in this solvent.

We have some evidence that similar chromatographic considerations may also operate in the case of other amino-acids, particularly L-[^{14}C]glutamic acid, and it would therefore appear advisable for the chromatography of amino-acids involving phenol as a solvent constituent to use an acid paper and to purify the phenol by distillation. Pretreatment of the paper with oxalic acid may also be beneficial as a result of binding any copper ions which may be present.

We wish to thank the Medical Research Council and the Empire Rheumatism Council for grants toward the cost of this work.

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PHARMACOLOGICAL CHARACTERIZATION OF THE OXYTOCIC PEPTIDES IN THE PITUITARY OF A MARINE TELEOST FISH (*POLLACHIUS VIRENS*)

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PITUITARY extracts from teleost fishes have been shown to contain at least two oxytocic substances[1,2]. In the pollock or pollack (*Pollachius virens*) one of these substances has been identified as arginine[8]-oxytocin (arginine vasotocin) by amino-acid analysis[2,3]. Arginine[8]-oxytocin has also been chemically characterized in neurohypophysial extracts from frog[4] and chicken[5]. Chromatographic[2] and pharmacological[2,6] evidence has been advanced for the presence of arginine[8]-oxytocin in the neurohypophysis of reptiles as well as in that of other amphibians and birds.

The second oxytocic principle found in pollock pituitary extracts was found to have many of the properties of oxytocin. Relative to its oxytocic potency, it showed very weak antidiuretic activity and, like oxytocin, had a much lower frog water balance activity than vasotocin[1]. When chromatographed on paper in butanol / acetic acid / water (4 : 1 : 5) (refs. 1 and 2) or eluted from a column of 'Amberlite *CG*-50' (ref. 2) it was found in the same position as oxytocin.

The 'second' oxytocic peptide in pollock pituitary extracts has now been compared with oxytocin ('Syntocinon', Sandoz) by further pharmacological tests. The sample of peptide used was prepared by paper chromatography of the material obtained from the region of oxytocin when pollock pituitary extract was eluted from a column of 'Amberlite *CG*-50' as in ref. 2. The active eluate from the paper chromatogram was freeze-dried and the resulting solid dissolved in 0·25 per cent acetic acid containing 0·5 per cent chlorbutol as preservative. This solution was used in all the biological assays described here.

The following tests were performed (the same batch of 'Syntocinon' = synthetic oxytocin, was used as standard throughout): (*a*) *Isolated rat uterus assay with and without magnesium:* Munsick[7] has shown that the action of many synthetic analogues of oxytocin on the isolated rat uterus is potentiated by the presence of magnesium in the suspension fluid. He showed also that the degree of potentiation varied from one peptide to the other. Accordingly, the unidentified pollock peptide was assayed on rat uteri suspended in either Munsick's[7] modified van Dyke-Hastings solution[8] without magnesium ions or in the same solution containing 0·5 m*M* Mg^{++}/l. The solution of the pollock peptide had a potency of 7 *U*/ml. in the absence of magnesium, but in the presence of this ion its potency was increased to

28 *U*/ml. Since 'Syntocinon' was the reference standard in both sets of assays, the increase in potency of the pollock peptide clearly differentiates it from oxytocin. (*b*) *Avian depressor assay*[9]: Estimations of the potency of the pollock peptide by this method gave a mean of 16·3 *U*/ml. compared with 7 *U*/ml. in assays on the isolated rat uterus without magnesium. (*c*) *Effect on sodium transport through the isolated frog skin* (natriferic activity[10]): unlike oxytocin, the unknown pollock peptide almost completely lacks the power to stimulate the sodium current produced by *Rana esculenta* skin *in vitro*. The mean natriferic potency was found to be 350 m*U*/ml. or 1/20 of the oxytocic activity. (*d*) *Effect on water movement through isolated frog bladder* (gravimetric assay[11,12]): as compared with oxytocin, the second pollock principle exhibited only a slight enhancing effect on the osmotic permeability of the *Rana esculenta* bladder. The mean activity was 750 m*U*/ml. or 1/9 of the oxytocic activity. (*e*) *Effect on water movement through the isolated frog skin* (volumetric assay[13]): The potency of the unidentified pollock peptide to enhance the net water flux through the isolated skin of *Rana esculenta* was very low, that is, about 350 m*U*/ml. or 1/20 of the oxytocic activity. (*f*) *Effect on water uptake by intact frogs*: in one *in vivo* experiment (21 animals) on *Rana esculenta*, the water-balance activity of the solution containing the unidentified pollock peptide was found to be less than 1 *U*/ml., that is, its potency in this test was less than 1/7 of that in assays on the rat uterus without magnesium ions. Low sensitivity of 'winter frogs' prevented further experiments.

The low potency of the unknown 'second' pollock oxytocic principle in all the amphibian tests is in striking contrast to the high potency shown by the 'first' oxytocic principle which has been chemically characterized as arginine[8]–oxytocin[2,3]. Compared with oxytocin, for example, the latter was found in a few preliminary experiments to be about 20 times more potent on the sodium transport across frog skin and 200 times more active on the water movement through the isolated frog bladder. It was already known to be about fifty times more active in the frog water balance test *in vivo*[2].

Fig. 1 shows the difference in the potencies of the two oxytocic principles found in pollock pituitary extracts with reference to their action on sodium transport across frog skin.

The effect of oxytocin on the renal water excretion of rats varies according to the conditions of the experiment[14,15], but it has been shown that the hormone has a weak antidiuretic action in intact rats with a high water load. Assayed against 'Pitressin', 'Syntocinon' was found to have an antidiuretic activity corresponding to 1·1 per cent of its oxytocic potency[15]. When the unidentified pollock peptide solution was compared with 'Pitressin', its antidiuretic action was equal to a mean of 13 m*U*/ml. or to 0·2 per cent of its oxytocic activity.

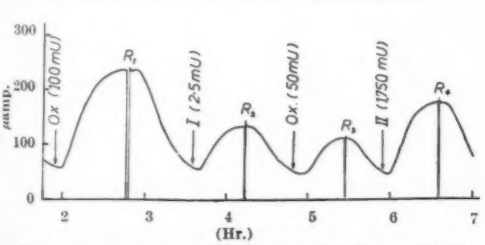

Fig. 1. Comparison of the effects of the two oxytocic pollock peptides and of oxytocin on the 'sodium current' (ref. 10) produced by frog skin. Ordinate: 'short circuited current' (in μamp.); abscissa: time, hr. Peptides added at arrows (in brackets: oxytocic = rat uterus activity). *Ox* = synthetic oxytocin; *R* = rinsing; I = the first oxytocic principle characterized as arginine[8]–oxytocin. Note that it was about 20 times *more* potent than oxytocin. II = The second oxytocic principle. Note that it was about 20 times *less* potent than oxytocin

In a few preliminary antidiuretic assays[16] on *Rana esculenta* the antidiuretic activity of the unknown pollock peptide was likewise found to be very weak, while the 'first' oxytocic peptide showed a high antidiuretic activity similar to that of arginine[8]– or lysine[8]–oxytocin.

Table 1 shows the 'pharmacological spectrum' of the solution containing the unidentified oxytocic peptide in pollock pituitary extracts. The standard in each of the assays shown was synthetic oxytocin ('Syntocinon'). If, therefore, the pollock peptide had been identical with oxytocin, the activity ratio (last column of the table) would have been unity in each type of assay. The fact that all the assays on amphibians gave activity ratios very much less than unity indicates that oxytocin, if present at all, could only have been present in very small amounts.

Table 1. POTENCY OF THE UNIDENTIFIED ('SECOND') OXYTOCIC POLLOCK PEPTIDE ASSAYED AGAINST OXYTOCIN ('SYNTOCINON', SANDOZ) (Note that, by definition, the activity ratios would be 1, had the unknown substance been oxytocin)

Assay method	Potency (*U*/ml.)	Ratio of activity to rat uterus (−Mg^{++}) potency
Rat uterus without Mg^{++}	7	1
Rat uterus with 0·5 m*M* Mg^{++}/l.	28	4
Chicken blood pressure	16·3	2·3
Natriferic activity (isolated frog skin)	0·35	0·05
Water-balance activity (isolated frog skin)	~0·35	~0·05
Water-balance activity (isolated frog bladder)	0·75	0·11
Water-balance activity (intact frog)	<1	<0·15

Until the unknown peptide is completely purified, it cannot be excluded that the preparation used in the present investigation contained other very similar peptides. However, the results presented are sufficient to demonstrate that teleost pituitary glands may contain not only arginine[8]–oxytocin (arginine vasotocin) but also another oxytocic peptide not found in the neurohypophysis of mammals. It would thus appear that the diversity of neurohypophysial active peptides in the vertebrate phylum is greater than hitherto assumed.

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EVIDENCE OF A 'HUNGER MOTIVATIONAL' SYSTEM IN THE LATERAL HYPOTHALAMUS OF THE RAT

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MUCH interest has recently been focused on the higher nervous control of food and water intake, particularly in the hypothalamic 'centres' concerned with the mechanisms of 'feeding' and 'satiety'. Several investigations[1–5] have tended to stress the importance of longitudinal hypothalamic fibre trajectories, especially the medial forebrain bundle, as being intimately involved in the motivation of feeding and drinking behaviour. Thus, Olds[4] classifies the lateral hypothalamic area along the medial forebrain bundle as a part of his 'reward' or 'pleasure' system, stimulation of which is 'preferred' by a hungry animal to actual feeding. Miller[6] has consistently found that stimulation of the lateral hypothalamic area in the perifornical area elicits feeding and drinking responses in satiated rats. His work indicates that the feeding and drinking areas are largely co-extensive with drinking responses slightly more prominent from the dorsal perifornical area. Miller has also shown that this stimulation evokes several properties of actual 'hunger' in that it would motivate learned food-seeking responses. Grossman[7], using the implanted cannula technique, has shown that injection of crystalline adrenergic substances into the peri- and supra-fornical areas elicits feeding in satiated animals and that acetylcholine injection caused water-sated animals to consume significantly more water. Furthermore, hungry rats would actually leave food, and drink water following acetylcholine injections.

In earlier experiments[8,9] I have shown that the medial forebrain bundle is not a crucial system in the effectuation of spontaneous feeding and drinking behaviour, since lesions in this bundle, singly and in combinations, anterior and/or posterior to the level of the 'feeding centre' did not chronically affect the intake of food or water. Only with lesions at the level of the ventromedial nuclei was the lateral hypothalamic syndrome of adipsia and aphagia produced, and these effects were radically different in nature depending on whether the lesions were in the mid-lateral or far-lateral hypothalamus. Mid-laterally lesioned animals showed a 'reversible' type of aphagia and adipsia, while far-laterally lesioned animals showed a more drastic 'failure' with no reversal tendencies even when carried 45 days on tube feedings. It was postulated that crossing fibre systems, mostly pallidofugal, were the critical elements in the organization of the feeding and drinking 'centres' since these were interrupted after far-lateral lesions but not after mid-lateral or perifornical lesions. The more drastic downhill course in the animals lesioned in the far-lateral hypothalamic area correlated well with degeneration in the pallidofugal fibre systems. Furthermore, it was shown afterwards[10] that lesions directed to the origins of these systems in the internal segments of the globi pallidi reproduced faithfully the far-lateral hypothalamic syndrome.

I have also shown[11] that the lateral hypothalamic area seems to be organized in a complex manner into a more medial 'hunger motivational' system and a far-lateral 'metabolic' system[11]. Animals lesioned medially and perifornically showed predominantly a 'motivational' failure toward food and water since this adipsic–aphagic state was easily reversible after forced feedings depending on the extent of the lesion. Some of these medially lesioned animals showed adipsic and aphagic reversal even when given special positively motivating foods such as chocolates, malts, etc., and it is now thought that all these animals are potentially reversible with proper post-operative support. On the other hand, far-laterally lesioned animals with pallidofugal fibre degeneration or animals with lesions in the pallida themselves are not so easily reversed. In my experience with 15 far-laterally lesioned rats supported by tube feeding, 5 died in spite of this treatment, 9 died of aphagia and adipsia after the tube feedings were discontinued (between 12 and 45 days post-operatively in the various animals), and only 1 showed reversal (resumption of spontaneous feeding and drinking behaviour) after 43 days of tube support. Of 9 animals lesioned in the pallida, 5 died during tube support while the remaining 4 reverted to adipsia and aphagia after tube removals (20–48 days) and died shortly thereafter. Five other animals lesioned in the pallida and not given tube support all died of aphagia and adipsia with food and water freely available. Thus, the far-laterally lesioned animals were not merely showing 'motivational inertia' to water and food but some additional defect not compensated for by actually delivering food and water to the gastro-intestinal tract. I have termed this a 'metabolic' failure as opposed to the purely 'motivational' failures of the mid-lateral and perifornically lesioned animals[10].

In the present experiments bipolar stainless-steel electrodes 0·20 mm. in diameter were implanted stereotaxically in the lateral and dorsal perifornical areas as well as in the far-lateral hypothalamic area. The stereotaxic co-ordinates used were: anterior planes 5·8–4·9 of DeGroot[12], parasagittal planes 1·0–1·7 for the perifornical implantations and 1·7–2·5 for the far-lateral implantations, and horizontal planes −1·5 to −3·0. This range of loci corresponded to several areas immediately around the fornix columns and in the areas of dispersion of pallidofugal fibre systems. Rectangular pulses of 0·2 msec. duration, 100 pulses per sec., were obtained from a 'Tektronix 162' waveform generator and a 161 pulse generator which drove a cathode follower output stage. The output from the cathode follower was fed to the electrodes through a General Radio isolation transformer. Current was monitored on a 'Tektronix 502' oscilloscope for each pulse series. The current generally used was 2 m.amp.

As a result of these stimulations additional support was added to the theory of the dual nature of the

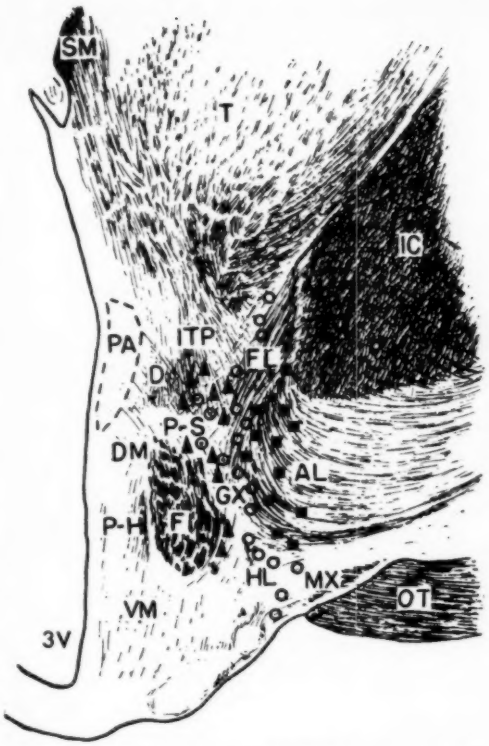

Fig. 1. Frontal section through the brain of the albino rat at anterior plane 5·8 of DeGroot with fibre systems indicated. Feeding responses to electrical stimulation and drug injection at points indicated by ▲; drinking responses to electrical stimulation and drug injection at points indicated by ○; no effects to either electrical stimulation or drug injection at points indicated by ○; feeding responses evoked by electrical and adrenergic stimulation at points indicated by ■. *SM*, stria medullaris; *T*, thalamus; *IC*, internal capsule; *PA*, paraventricular nucleus; *ITP*, inferior thalamic peduncle; *D*, dorsal hypothalamic area; *FL*, fasciculus lenticularis of Forel; *P-S*, paraventriculo-supraoptic fibres; *DM*, dorsomedial hypothalamic nucleus; *P-H*, pallido-hypothalamic fibres; *F*, fornix; *GX*, dorsal supraoptic commissure, pars dorsalis (Ganser); *AL*, ansa lenticularis; *HL*, lateral hypothalamic area; *VM*, ventromedial hypothalamic nucleus; *OT*, optic tract; *MX*, dorsal supraoptic commissure, pars ventralis (Meynert); *3V*, third ventricle

organization of the 'feeding centre' and in the first part of the present work confirmation of Miller's[6] work has been obtained. In 8 animals electrical stimulation of the dorsal and lateral perifornical areas has resulted in selective feeding or drinking responses in satiated states. The areas stimulated to produce feeding and drinking behaviour are shown in Figs. 1 and 2, which represent the two sterotaxic frontal planes utilized. Both these figures show that feeding and drinking responses were obtained mostly from the dorsal and lateral perifornical areas and that these response areas were distinctly separated from other response areas in the far-lateral hypothalamic area by regions of no response. However, after lesioning the medial forebrain bundle anterior and posterior to the level of the 'feeding centre' and repeating these perifornical stimulations (same animals with electrodes in place) no feeding or drinking responses were obtained in any of the animals. On the other hand, it was previously shown[11] that sated animals stimulated in the far-lateral hypothalamic area would feed even with the medial forebrain bundle lesioned. These far-lateral stimulated animals would not, however, run an electrical barrier to press a lever for food. Before lesioning the medial forebrain bundle the animals stimulated in the far-lateral hypothalamic areas would both feed in the sated state and run the electrical barrier to press a lever for food. This evidence certainly indicates essential differences in behaviour produced by the mid-lateral (or perifornical) stimulation as opposed to far-lateral stimulation. Figs. 1 and 2 also indicate the areas in the far-lateral hypothalamus stimulated in the present experiments. Stimulations in the areas shown provoked feeding in sated animals regardless of whether or not the medial forebrain bundle was intact.

Also in the present experiments we compared Grossman's studies as to the effects of injecting adrenergic and cholinergic drugs into the dorsal and lateral perifornical areas with the effects seen after these same substances were injected into parts of the far-lateral hypothalamus. Selective feeding and drinking responses were obtained from the perifornical areas, the adrenergic substances producing feeding in the sated state and the cholinergic substances 'pure' drinking responses. In these animals (with injection needles in place), after section of the medial forebrain bundle anterior and posterior to the level of the 'feeding centre', no feeding or drinking responses could be evoked by injection of these drugs into the perifornical areas. In another group of animals injection of adrenergic and cholinergic drugs into the far-lateral hypothalamus produced somewhat different results. Adrenergic drugs did

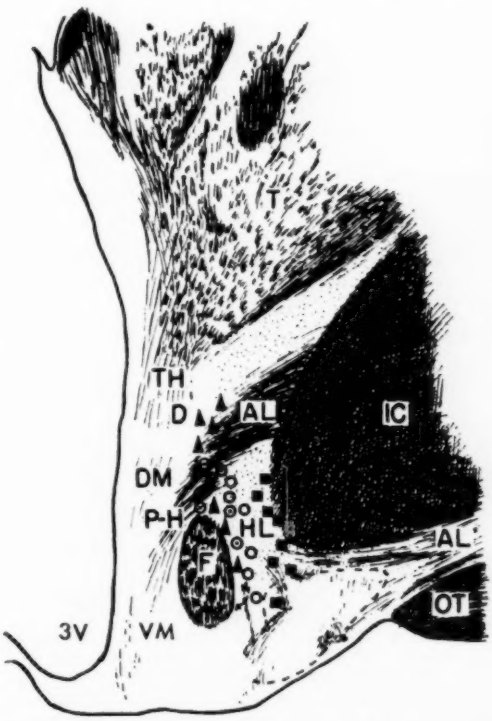

Fig. 2. Frontal section through the brain of the albino rat at anterior plane 4·9 with fibre systems indicated. Feeding and drinking responses designated as in Fig. 1. Notations for fibre systems and nuclei as in Fig. 1. *TH*, thalamo-hypothalamic fibres

XUM

provoke feeding in the sated state, but no 'pure' drinking responses could be obtained with cholinergic substances. After section of the medial forebrain bundle in this far-lateral group, however, feeding responses in the sated state were still easily obtainable following injection of adrenergic materials. These feeding responses were obtained from the same areas of the far-lateral hypothalamus as those that responded to electrical stimulation (Figs. 1 and 2). It is clearly seen from these figures that the critical areas are directly in the dispersion fields of the pallidofugal fibre systems (ansa lenticularis and lenticular fasciculus of Forel).

It is concluded that the mid-lateral and perifornical portions of the lateral hypothalamus contain 'hunger motivational' systems important in food drives. Intactness of the medial forebrain bundle seems necessary for this system to function properly since this system can only be activated (by stimulation or drug injection) in sated animals to produce further eating (or drinking) if the medial forebrain bundle is intact. Oddly enough, stimulation farther laterally in the far-lateral hypothalamus will cause sated animals to feed even when the medial forebrain bundle is lesioned; on the other hand, certain learned food-seeking responses are not provoked by far-lateral stimulation when the medial forebrain bundle is disjoined. It thus seems that the far-lateral mechanism is the more 'basic' feeding system and is mostly dependent on activation of pallidofugal fibre trajectories having primary influences perhaps on neuro-metabolic regulations. The medical and perifornical systems seem to be purely 'motivational' in nature, that is, are concerned only with the 'urge' of the animal to approach food and water.

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EVIDENCE FOR A NEW ENZYME CONNECTED WITH PROTEIN SYNTHESIS

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THE enzymic reaction which incorporates amino-acids into soluble RNA has been so extensively studied that only review references can be given here[1–4]. The first step involves the formation of an enzyme-bound amino-acid adenylate compound. The second step is the transfer of the activated amino-acid to the 2′- or 3′-hydroxyl of the terminal adenosine residue in the soluble RNA. The overall reaction is summarized by the following formula:

$$\text{amino-acid}_1 + \text{adenosine triphosphate} + \text{soluble RNA}_1 \overset{\text{enzyme}_1}{\rightleftarrows} (\text{amino-acid}_1)\text{-RNA}_1 + \text{adenosine monophosphate} + \text{inorganic pyrophosphate} \quad (1)$$

Most probably there is at least one specific acceptor RNA molecule and one specific enzyme for each amino-acid, and the same enzyme ('the activating enzyme') should be able to carry out the activation and the transfer to the soluble RNA. Purifications of more than a dozen activating enzymes from various sources have been reported[1–4]; but so far most of these preparations have not been obtained in a pure and homogeneous form. It may also be significant that these enzymes show very low figures for turn-over number when such calculations are possible for the overall reaction (1).

In this article we are recording a few experiments which may contribute additional information about reaction (1). We have prepared two partially purified enzymes: the arginine activating enzyme from *Escherichia coli*[5] and the methionine activating enzyme from yeast[6]. The methionine incorporating activity of these two enzymes in combination has been found to be up to twelve times as large as the sum of their separate activities. This finding, together with kinetic observations and metal ion effects, is taken as evidence for the existence of a new enzyme connected with reaction (1).

The RNA was prepared from bacteria by extraction of the whole cells with 90 per cent phenol followed by alcohol precipitation of the total RNA from the water phase. The soluble RNA was extracted from the precipitate by cold *M* sodium chloride[7] and further purified by precipitation with lanthanum nitrate[8]. The lanthanum salt of soluble RNA was dissolved in 0·5 *M* ethylenediamine tetraacetic acid of *p*H 4·7 and finally dialysed against distilled water for 15 hr. The activity of the activating enzymes was measured as the ability to incorporate C-14 amino-acids into soluble RNA. The assay volume was either 0·5 or 0·2 ml. and the reaction mixture contained varying amounts of soluble RNA and activating enzyme, 0·11 m*M* C-14 L-amino-acid, 2 m*M* adenosine triphosphate, 2 m*M* glutathione, 4 m*M* magnesium chloride and 0·1 *M* *tris*-maleate buffer, all adjusted to *p*H 7·0. The reaction was terminated by precipitation with 0·01 *M* lanthanum nitrate in 0·5 *M* perchloric acid. Carrier RNA was added, the precipitate was washed and the radioactivity determined with a windowless gas-flow counter as described in detail elsewhere[8].

During the study of the partially purified arginine activating enzyme, it was found to be gradually inactivated at 0° and *p*H 4·1. The time-dependence

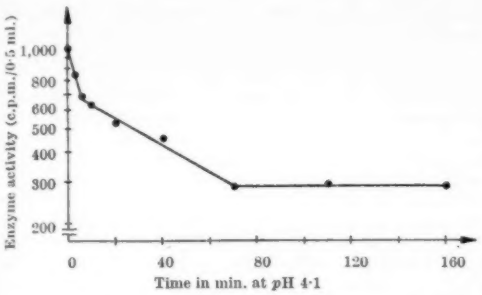

Fig. 1. Denaturation of arginine activating enzyme at 0° and pH 4·1. Enzyme activity measured as C-14 arginine–RNA formed in 15 min. at 37°. Assay volume 0·5 ml. with 4·3 μgm./ml. of enzyme and 4·0 mgm./ml. of soluble RNA. The activity is plotted on a logarithmic scale and is given in c.p.m. corrected for background and the mean blank, but not for self absorption

for this denaturation is shown in Fig. 1. The assay was performed with an enzyme concentration of 4·3 μgm./ml. and a soluble RNA content of 4 mgm./ml., conditions which make the enzyme rate-limiting for more than 15 min. Three blanks were used: one with enzyme heated to 70° for 10 min. gave 160 c.p.m., one with 0·02 *M* ethylenediamine tetraacetic acid pH 6·8 gave 150 c.p.m., and one with 0·002 *M* *p*-chloromercuribenzoate gave 165 c.p.m. The mean value of these blanks has been subtracted from the data given. Fig. 1 shows that about 25 per cent of the enzyme activity is stable at pH 4·1 and that the denaturation cannot be a single first-order reaction, but might be the sum of two such reactions. It has been checked that a partially denatured sample cannot be reactivated by standing at pH 7·0.

Berg and his associates[3] have compared the incorporation of methionine into *E. coli* soluble RNA when using methionine activating enzymes from the same source and from yeast. They found the bacterial enzyme to incorporate more than twice as much as the yeast enzyme. This result has been interpreted as a heterogeneity in the methionine accepting RNA[3,4]. We have used the working hypothesis that Berg's observation could be related to the results given in Fig. 1 and that such systems might permit the assay of different enzymes involved in the transferring of the same amino-acid to the soluble RNA. An attempt was thus made to purify the arginine activating enzyme from yeast (*Saccharomyces cerevisiae* C836) and it was found that the preparation *AS*-1 of the methionine activating enzyme described by Berg[5] was a potent source of enzyme for the incorporation of both arginine and methionine as seen from Table 1. It can also be seen that the two enzyme preparations have almost the same specific activity for the methionine incorporation, but that in combination they have about 13 times higher specific activity.

Table 1. Rate Constants and Specific Activities for the Enzyme Preparations

Incorporation in soluble RNA with	C-14 L-arginine		C-14 L-methionine	
Enzyme preparation	Rate in c.p.m./min.	Spec. act.	Rate in c.p.m./min.	Spec. act.
Arginine activating enzyme from *E. coli*	72·2	20·2	1·0	0·014
Methionine activating enzyme from yeast	7·5	0·014	12·8	0·016
Methionine activating enzyme + arginine activating enzyme	94·4	—	167	0·210
Methionine activating enzyme + pH 4 treated arginine activating enzyme	—	—	25·6	0·027

The experiments with both enzymes in combination were performed with the same amount of the same enzyme preparations which were tested alone. The experiment in the last line was carried out with another preparation of methionine activating enzyme with a specific activity of 0·028. The rate constants are derived from the initial slopes of time-curves using 0·2 ml. assay volume. The specific activities are given in mμmoles of amino-acid incorporated at 27° (in an excess of soluble RNA) per min. and per mgm. of protein.

The effect of the combined enzyme preparations has been further examined in kinetic experiments of various types. Fig. 2 shows two parallel experiments when arginine activating enzyme was added to a reaction with methionine activating enzyme. The result is a significant increase in the formation of methionine–RNA, while the control experiment with arginine activating enzyme shows only a minor incorporation. The addition of more methionine activating enzyme after 60 min. does also give an increased formation of methionine–RNA indicating that soluble RNA does not become rate-limiting during the experiment. One of the experiments in Fig. 2 was carried out with C-14-methyl labelled L-methionine (represented by circles) the other with C-14-(C2) labelled DL-methionine (represented by squares); both obtained from California Corporation for Biochemical

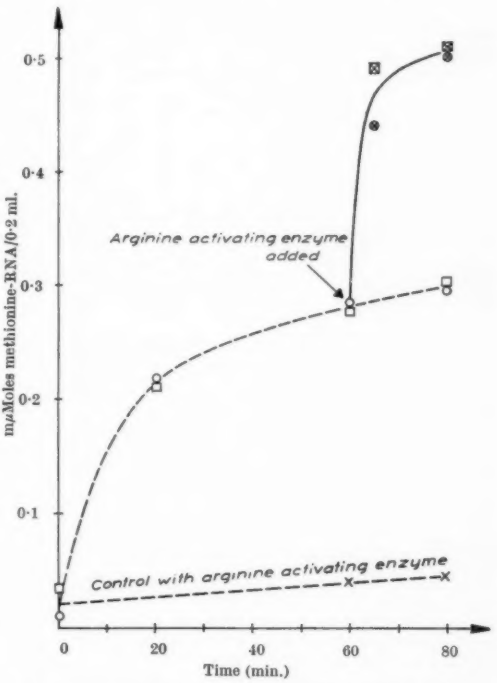

Fig. 2. Two parallel kinetic experiments at 27° with 1·24 mgm./ml. of methionine activating enzyme and 3·1 mgm./ml. of soluble RNA. Arginine activating enzyme (final conc. 0·17 mgm./ml.) was added to the reaction mixture after 60 min. Aliquots of 0·2 ml. were withdrawn at various times and assayed for C-14 methionine-RNA. Circles represent L-methionine with methyl-C-14, squares DL-methionine with (C2)-C-14. The crosses represent an experiment with 0·098 mgm./ml. of arginine activating enzyme, 2·6 mgm./ml. of soluble RNA and L-methionine with methyl-C-14

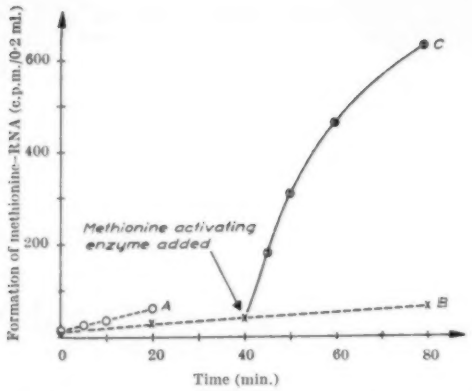

Fig. 3. Kinetic experiment at 27° showing the formation of methionine–RNA using 0·75 mgm./ml. of methionine activating enzyme (curve *A*) and using 68 μgm./ml. of arginine activating enzyme (curve *B*). After 40 min. was added to the latter reaction mixture methionine activating enzyme to a final concentration of 0·75 mgm./ml. (curve *C*). The concentration of soluble RNA was 4·5 mgm./ml. for curves *A* and *B*, 4·1 mgm./ml. for curve *C*. Assay volume 0·2 ml. The activity is given in c.p.m. and corrected only for background

Research, Los Angeles, 63. The agreement between the two experiments rules out a transmethylation reaction.

Both the enzyme preparations were stored at − 18° C. with 50 per cent ethylene glycol as anti-freeze. In these conditions, the arginine activating enzyme has been stable for 7 months, while the methionine activating enzyme gradually has become less active. Fig. 3 shows the time-curves for the formation of methionine–RNA using a somewhat inactivated preparation of methionine activating enzyme (curve *A*) and a normal preparation of arginine activating enzyme (curve *B*). After 40 min. the same amount of the same preparation of methionine activating enzyme was added to the latter reaction mixture as was used for curve *A*. The suddenly increased formation of methionine–RNA obtained by the combined enzymes (curve *C*) corresponds to about a nine-fold increase in specific activity.

Most enzymic reactions are characterized by the fact that the enzyme concentration is rate-limiting in the beginning, while the substrate concentration becomes limiting towards the end of the reaction. Curves corresponding to this type of kinetics are also found for the formation of arginine–RNA using the arginine activating enzyme and for the formation of methionine–RNA using both the arginine and the methionine activating enzymes together. However, the curve obtained with the methionine activating enzyme alone is approximately logarithmic with time (as in Fig. 2) and similar curves are obtained also with arginine activating enzyme which has been kept at 0° and *p*H 4·1 for 80 min. Such logarithmic curves may be expected when a rate-limiting component is disappearing in a first-order reaction.

Some factors which could be expected to influence the rate or the equilibrium position of reaction (1) have been tested for their possible effects. Both the enzyme fractions have been pre-incubated at 27° for 80 min. without any detectable change of activity. The addition of crystalline pyrophosphatase[10] and purified venom 5′-nucleotidase[11] had no influence on the course of the reaction. An instability of one of the enzyme fractions or a trapping of the reaction products can thus be eliminated as interpretations.

However, it was found that in the presence of magnesium, the addition of calcium, cobalt or manganese ions has a pronounced stimulating effect on the methionine activating enzyme preparation, but a slight inhibitory action on the preparation of arginine activating enzyme. On the other hand, the addition of ashed preparations of the respective enzymes had scarcely any effect on the reaction. Thus the sudden increase in the formation of methionine–RNA observed with both enzymes together (Figs. 2 and 3) cannot be a metal-ion effect.

All the results reported here could be explained by the following hypothesis, which is based on two assumptions. (*a*) The activating enzyme is consumed during the course of the reaction (1). It could be that the reaction produces a chemical change in the enzyme or that 'the enzyme-substrate compound' formed is not split into 'product and enzyme'. The methods used do not distinguish between these alternatives, but both may be expected to give logarithmic time-dependences. (*b*) A second enzyme exists which is capable of regenerating the methionine, as well as the arginine activating enzyme. This 'regenerating enzyme' is present in an excess in the arginine activating enzyme preparation, which cannot be regarded as highly purified despite its high specific activity (see Table 1). The methionine activating enzyme was purified about fifty-fold[6], but has probably been freed of the 'regenerating enzyme', which explains both its relatively low specific activity and the stimulation by the arginine activating enzyme (Table 1).

The 'regenerating enzyme' may have its maximum activity with magnesium ions, which explains the general agreement[1–4] about the requirement for this metal ion. We have determined that the arginine activating enzyme has its maximum activity with 0·01 *M* magnesium but that about 50 per cent activity is obtained with manganese. The alanine activating enzyme from pig liver cytoplasm requires cobalt[12], and the tyrosine activating enzyme from rat liver has recently been shown to require potassium[13]. Other activating enzymes may show optimum activity with other metal ions such as calcium, iron and manganese, all of which are known to be present in RNA preparations[14], but which are removed by our treatment with ethylenediamine tetraacetic acid. Preliminary experiments have indicated that besides methionine this would be the case with the enzyme for phenylalanine.

The difference between the methionine activating enzymes from yeast and from bacteria observed by Berg *et al.*[9] can thus be explained by assuming that their preparation of the bacterial enzyme contains the 'regenerating enzyme' and that this is absent in the preparation of the yeast enzyme. Zillig *et al.*[15] have reported that the amount of leucyl–RNA which can be formed by their '*p*H 4 enzyme' is directly proportional to the amount of enzyme used, and suggest that the normal reaction product is a complex of amino acyl soluble RNA and enzyme. Our interpretation would be that the 'regenerating enzyme' is denatured by the *p*H 4·5 precipitation used by these authors, as well as in our *p*H 4·1 experiments with the arginine activating enzyme (Fig. 1 and Table 1). Such a denaturation would explain why enzyme preparations subject to the acid treatment show the anomalous dependence on enzyme observed.

In conclusion, we feel that our results are in agreement with the previous findings of Berg's and Zillig's groups, and that together with our own data they support the hypothesis of the existence of a 'regenerating enzyme' coupled to the activating enzymes which catalyse reaction (1). Such a coupling could be a powerful control mechanism, but speculations about its possible function *in vivo* may await further experimental data. It is a consequence of our hypothesis that in the presence of soluble RNA reliable measurements with activating enzymes cannot be performed without an excess of 'regenerating enzyme'.

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Note added in proof. Dr. Paul Berg has kindly informed us that soluble RNA from bacteria can be obtained in a form in which a fraction of the chains lacks the adenosine end-group. Consequently the effect of the arginine activating enzyme preparation could be due to incorporation of adenosine monophosphate, which would increase the number of acceptor sites for methionine. However, preincubation of our soluble RNA with the arginine-activating enzyme preparation and adenosine triphosphate has failed to demonstrate any increase in the number of acceptor sites for methionine.

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PHOTOPHOSPHORYLATION AND THE HILL REACTION

THE rate of the Hill reaction has been reported to be stimulated strongly by concomitant photophosphorylation, that is, by synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and orthophosphate[1-4]. Warburg *et al.*[5] have pointed out that this stimulation of the rate of photoreduction is an effect of *p*H, but the warning appears to have gone unheeded. Most recently, for example, Good[6] has used such a stimulation to distinguish between phosphorylating and non-phosphorylating electron transport; he suggests that the latter should be subtracted from the former to obtain the true ratio of micromoles ATP formed to electron pairs transferred (P/2e ratio), and concludes that this value is 2. Good cites a similar value of 2 reported by Krogmann and Vennesland[7] for the stoichiometry of oxidative photophosphorylation. We must point out, however, that the assumptions underlying the latter calculation are quite different from those made by Good, and may or may not be correct. At the same time, our own work on the dependence on *p*H of the Hill reaction leads us to the conclusion that Warburg is correct in attributing rate stimulation by associated phosphorylation to effects of *p*H.

Punnett[8] has noted that freshly prepared chloroplasts photoreduce ferricyanide most rapidly at a rather alkaline *p*H. With spinach chloroplasts freshly prepared in 0·35 *M* sodium chloride, the *p*H optimum we observe is generally greater than *p*H 8. The rates at the maximum, in saturating light, range up to 600 μmoles per mgm. chlorophyll per hr. at 15° C., and may be as much as four times faster than the rates measured at *p*H 7 with the same chloroplast preparations. Fig. 1 shows the dependence on *p*H of photoreduction and photophosphorylation in 0·05 *M* *tris*(hydroxymethyl)aminomethane buffer, where the abscissa represents the initial *p*H of the reagents employed. Reactions were carried out in 25-ml. conical flasks incubated at 15° C. with air as the gas phase. Each vessel contained in micromoles: *tris*(hydroxymethyl)aminomethane, 100; potassium ferricyanide, 10; magnesium chloride, 10; and, where indicated, potassium phosphate and adenosine diphosphate, 3·4 in a final volume of 2·0 ml. *Tris*, phosphate, and adenosine diphosphate solutions were adjusted to the indicated *p*H with the use of hydrochloric acid and sodium hydroxide and a Leeds and Northrup *p*H meter. Chloroplasts, freshly prepared in 0·35 *M* sodium chloride and used without prior washing, containing 113 μgm. of chlorophyll were added to the vessels at zero time. The reactions were initiated and terminated by turning the light on and off; illumination was from below for 4 min., with shaking, and with white light of about 5,000 ft.-candles. Reaction mixtures were deproteinized by adding 0·50 ml. of 20 per cent (w/v) trichloracetic acid and centrifuging. Residual ferricyanide was determined on an aliquot of the supernatant by direct reading at 400 mμ in the Beckman *B* spectrophotometer. Residual orthosphosphate was determined on a separate aliquot by the method of Fiske and Subbarow[9]; the reduced phosphomolybdate complex was read in a Lumetron colorimeter with a 650-mμ filter, after centrifuging to remove the precipitate of ferrocyanide–molybdate. Chlorophyll was determined by Arnon's method[10].

The upper curves represent ferricyanide photoreduction in the presence of ADP and orthophosphate (solid circles) and in the absence of both ADP and orthophosphate (open circles). The largest effect of these reagents (20 per cent stimulation) is seen at the lowest *p*H. This effect diminishes as the initial *p*H approaches the *pK* of the buffer (8·2), and it could be enhanced by the use of lower buffer concentrations. This behaviour can be accounted for on the basis of the formation of hydrogen ions during ferricyanide photoreduction (equation 1) and the consumption of hydrogen ions during the synthesis of ATP (equation 2):

$$2Fe(CN)_6^{3-} + H_2O \rightarrow 2H^+ + 2Fe(CN)_6^{4-} + \tfrac{1}{2}O_2 \quad (1)$$

$$ADP^{3-} + HPO_4^{2-} + H^+ \rightarrow ATP^{4-} + H_2O \quad (2)$$

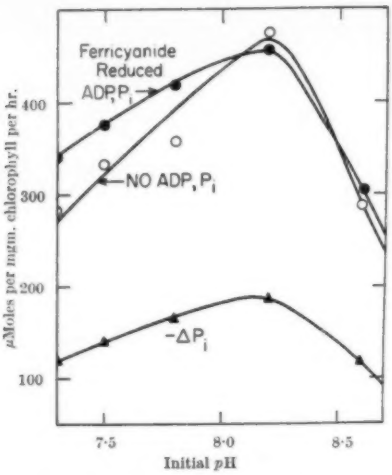

Fig. 1. Dependence on *p*H of ferricyanide photoreduction and phosphate uptake by spinach chloroplasts in the presence and absence of a phosphorylation system

The lower curve of Fig. 1 shows the rate of phosphate consumption accompanying ferricyanide photoreduction in the presence of ADP and phosphate. It should be noted that the rate of phosphorylation here is proportional to the rate of ferricyanide reduction and that the P/2*e* ratio is about 0·8 throughout the *p*H range. If we were to apply the correction suggested by Good[6] for non-phosphorylating electron transport in the calculation of the P/2*e* ratio, values for the latter in this experiment would range from four to infinity.

The reaction conditions detailed in Fig. 1 have been employed extensively in our laboratory for the study of the stoichiometry of the Hill reaction and associated photophosphorylation. An examination of published results from other laboratories[4] shows that very large stimulations of ferricyanide photoreduction by ADP have been obtained with rather different reaction mixtures which contain ATP, higher concentrations of phosphate and lower concentrations of chlorophyll and ferricyanide, assayed with shorter illumination periods. Table 1 shows that we can also obtain a stimulation of the Hill reaction-rate under these conditions, even in well-buffered systems. The correct interpretation of the nucleotide effects shown in Table 1 is not entirely clear, and would seem to require further investigation. But from numerous experiments such as that illustrated in Fig. 1, it is clear that the addition of ADP and orthophosphate to adequately buffered chloroplast suspensions can elicit a good rate of photophosphorylation without appreciable stimulation of the rate of ferricyanide photoreduction. Thus we are in agreement with Warburg's conclusion, namely, that photophosphorylation can occur as a result of the Hill reaction, but that the rate of the latter is independent of the ATP synthesis which may or may not accompany it.

Table 1. Effect of ADP and ATP on Ferricyanide Photoreduction by Spinach Chloroplasts

The reaction mixture contained in μmoles: *tris*(hydroxymethyl)-aminomethane, 82; potassium phosphate, 68; potassium ferricyanide, 2; magnesium chloride, 10; sodium chloride, 70; in a final volume of 3·0 ml., at *p*H 7·8, and chloroplasts containing 74 μgm. chlorophyll. Illumination was for 2 min.; other conditions and assay as described for the experiment in Fig. 1

Additions	Ferricyanide photoreduction (μmoles per mgm. chlorophyll per hr.)
None	282
1 μmole ADP	425
0·5 μmole ATP	172
1 μmole ADP + 0·5 μmole ATP	403

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The following considerations compel me to reject the *p*H theory:

(1) There is always much less formation of hydrogen ion associated with the reduction of flavins than with the reduction of ferricyanide. When flavins function catalytically in the reduction of oxygen, as in my usual test system, there is no hydrogen ion formation at all. Nevertheless, I find that the reduction of flavin mononucleotide is stimulated to the same extent as the reduction of ferricyanide by the presence of the phosphorylating system.

(2) The acid consumed in the phosphorylation of adenosine diphosphate reappears when the resulting adenosine triphosphate is used in the synthesis of glucose-6-phosphate. Yet the stimulation of ferricyanide and flavin reduction which is associated with the phosphorylation is independent of the presence or absence of hexokinase and glucose.

(3) Chloroplasts stored below *p*H 7·0 rapidly lose all ability to respond to the components of the phosphorylating system. These aged chloroplasts continue for a long time to reduce ferricyanide in the absence of the phosphorylating system at the same rate as freshly prepared chloroplasts. Consequently, the phosphorylating and non-phosphorylating electron transport systems must differ in at least one labile reaction step. This fact precludes the possibility that the rate-stimulation observed during phosphorylation represents the effect of improved conditions, *p*H or other, on a single process.

(4) The evidence presented by Stiller and Vennesland in support of Warburg's *p*H hypothesis contradicts the experience of most workers. I have carefully considered buffering problems and have established satisfactory buffer-levels by exhaustive experimentation without encountering phenomena of the kind described by the Chicago group. I believe that the high rates they attribute to "adequate buffering" are, in fact, an expression of the hitherto unrecognized uncoupling effect of high concentrations of salts. This effect of salts on chloroplasts will be documented in detail elsewhere. Chloroplasts I have

used exhibit the familiar pattern of behaviour regardless of the alkalinity and the concentration of the buffer when they are suspended in media low in ions; the complete phosphorylating system always causes large increases in the rate of ferricyanide reduction and there is always a quantitative relationship between the increase of rate and the amount of adenosine triphosphate formed. On the other hand, these same chloroplasts in 0·35 *M* sodium chloride are similar to, but not identical with, the Stiller–Vennesland chloroplasts. The condition described by Stiller and Vennesland, phosphorylation without stimulation, would be kinetically reasonable if their chloroplasts were both partially uncoupled and partially inhibited (as mine seem to be in 0·35 *M* sodium chloride). Thus the uncoupling might be sufficient to allow rates to rise to the maximum of a partially inhibited process which would then become rate-limiting. Yet this amount of uncoupling might still permit considerable phosphorylation; hence there is phosphorylation but no increase in the rate of electron transfer. However, the effects of salts on chloroplasts are very complex, with many as yet unexplored ionic interactions. Consequently there is little point in attempting a detailed interpretation of the Stiller–Vennesland observations at present.

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Stiller and Vennesland (first communication) present a timely warning with respect to the interpretation of experiments measuring the rate of the Hill reaction when ferricyanide or other redox dyes are reduced. I agree that the *p*H of the reaction mixture can fall during the course of the reaction if insufficient buffer is present; and further that a concomitant phosphorylation of ADP will prevent this fall. My measurements also show a fairly sharp optimum at about *p*H 8; thus a downward drift in *p*H would cause a lower rate of dye reduction or of oxygen evolution.

It is another question, however, whether or not this sort of artefact is actually responsible for previously reported phenomena[1–3]; and whether in the complete absence of a drift of *p*H the rate of the Hill reaction is stimulated due to an accompanying phosphorylation reaction.

In our first account of stimulation of rate by adding ADP, magnesium and orthophosphate[1], which confirmed the earlier observation of Arnon *et al.*[4]; and also in our published work since then[2,3], the reactions were run at room temperature, in 3 ml. total volume containing either 0·030 or 0·040 mgm. of chlorophyll, 40 μmoles of *tris* buffer at *p*H 8, 70 μmoles of sodium chloride and 1·5 μmoles of ferricyanide. (Unfortunately the original tables (ref. 1) omitted mention of the *tris*; its presence was indicated only indirectly by reference to earlier papers.) The illumination period was 2 min., so that initial rates only have been measured. The control reactions, to which no other reagents were added, reduced at most one-third of the ferricyanide present, or 0·5 μmole. It seems quite unlikely that the equivalent amount of hydrogen ions generated could have lowered the *p*H significantly in the presence of 40 μmoles of *tris* at *p*H 8. A recheck of the same reaction conditions shows at most a *p*H fall of 0·1 unit in the control reactions. This could not be enough to account for more than a minor share of the difference in rate between the phosphorylating and non-phosphorylating reactions.

I have re-examined the rate of ferricyanide reduction as a function of *p*H, using 150 μmoles of *tris* buffer in 3 ml. volume as suggested by Stiller and Vennesland, in order to eliminate any possible drift of *p*H. The *p*H's of the reaction mixtures were all measured after illumination ended as a further precaution against undetected drifting. The results shown in Fig. 1 are from a typical experiment out of five repetitions. All points represent the average of duplicate samples, containing, in 3 ml. total volume, 0·040 mgm. of chlorophyll, 70 μmoles of sodium chloride, 150 μmoles of *tris* at an appropriate *p*H and 1·5 μmoles of potassium ferricyanide. Where indicated to be present, there were 30 μmoles of phosphate adjusted to the same *p*H as that of the *tris*, 10 μmoles of magnesium chloride, 0·2 μmole of ATP or 1·5 μmoles of ADP. The reaction mixtures were illuminated in small test-tubes for 2 min. at room temperature, at 5,000 ft.-candles (the source was an incandescent lamp with 5 cm. of water filter between the lamp and the reactions). The *p*H's shown were those of the reaction mixtures, determined on each sample after the reaction was ended. Following this, the mixtures were killed by adding 0·3 ml. of 20 per cent trichloracetic acid, the proteins removed by centrifugation, and optical density of the supernatant solution measured at 420 mμ in a Beckman spectrophotometer. The ferricyanide reduced was measured as the change in optical density from that of zero-time control, and no appreciable change could be seen without illumination. The chloroplasts were prepared by grinding spinach leaves in a buffer containing 0·40 *M* sucrose, 0·02 *M tris* buffer and 0·01 *M* sodium chloride at *p*H 8·0. They were centrifuged once, resuspended in 0·40 *M* sucrose/0·03 *M* sodium chloride, and used without washing. The entire *p*H series was accomplished within an hour and a half after grinding the leaves, the higher *p*H's being measured first.

The results show that reactions with and without a simultaneous phosphorylation have a similar response to *p*H; but at any one *p*H the rate is usually doubled owing to addition of ADP. As before[1],

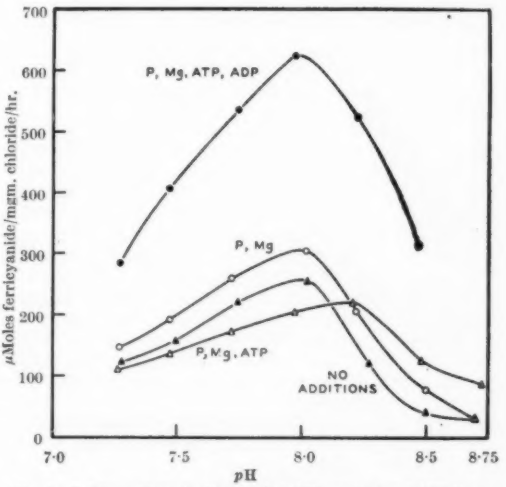

Fig. 1. Ferricyanide reduction by the Hill reaction as a function of *p*H of the reaction mixture

ATP at low concentrations has an inhibitory effect, so that if ATP, magnesium ions and orthophosphate serve as a control, the rate may be tripled owing to addition of ADP. These results correspond to those of Table 1 in the communication by Stiller and Vennesland. Although I agree that the interpretation of these results is not clear if they stand alone, we have previously[1] presented results from various other types of experiments, all of which supported the concept that the actual process of simultaneous phosphorylation was responsible for the observed increase of rate.

It was recently discovered that the rate of reduction of triphosphopyridine nucleotide (TPN) is stimulated by adding all three reagents required for phosphorylation (ref. 5, and San Pietro and Keister, in preparation). Although the extent of such stimulation may be four-fold, or even more, a *p*H drift of no more than 0·03 unit can be seen under the reaction conditions used (San Pietro and Keister, personal communication). Furthermore, the *p*H response for reduction of TPN appears to be considerably less steep than that for ferricyanide reduction.

In view of all these results, I must take exception to the generalization implicit in the statement of Stiller and Vennesland attributing ". . . rate stimulation by associated phosphorylation to effects of *p*H". The statement obviously cannot apply to the results obtained under the conditions of my Fig. 1, or of Table 1 of Stiller and Vennesland. This interpretation of stimulation of rate has a chance of being significant only when the Hill reaction is allowed to proceed for longer periods of time, generating major amounts of hydrogen ions (as is usual when manometric procedures are used, for example). It should be noticed that in Fig. 1 of Stiller and Vennesland the 4-min. reaction period with a fairly high chlorophyll-level leads to the generation of approximately 4 μmoles of hydrogen ions, rather than the 0·5 μmole or less under my conditions.

It is always possible that under differing conditions a particular phenomenon may not appear. Using the reaction conditions as described by Stiller and Vennesland for their Fig. 1, I have confirmed their observation that the Hill reaction-rate is unchanged (or shows no more than a 5–10 per cent stimulation) due to a simultaneous phosphorylation. I have found that one condition contributing significantly to the smaller effect is the low concentration of phosphate employed ($1{\cdot}67 \times 10^{-3}$ *M*) compared with that needed for a maximal rate of phosphorylation[1,6]. It seems to me reasonable that in a complex system with a very significant basal level at all times, close to optimal conditions will be required for the induced reactions to become apparent. The possible role of other factors in diminishing the rate-stimulation by ADP, magnesium ions and orthophosphate is at present under investigation.

As Stiller and Vennesland point out, the variations possible in relative rates between phosphorylating and non-phosphorylating reactions make the calculation of the P/2e ratio after subtracting the basal rate an extremely questionable operation. It seems to me that this same demonstrable variation also invalidates the generalization implicit in the statement of Stiller and Vennesland, ". . . that photophosphorylation can occur as a result of the Hill reaction, but that the rate of the latter is independent of ATP synthesis which may or may not accompany it".

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BIOLOGY OF REPRODUCTION IN *RUBUS*

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APOMIXIS has, in the past, been frequently defined to include diverse types of vegetative reproduction such as bulbil formation and vegetative proliferation[1]. In this account the use of the term is restricted to embryo formation from a cell which has not undergone meiosis and has not been fertilized. It is usually assumed that many polyploid species of *Rubus* reproduce either by facultative or obligate apomixis. Thus *R. laciniatus* ($2n = 4x = 28$) is believed to be an example of a facultative apomict while *R. caesius* ($2n = 28$) is reported as being entirely apomictic. In *Rubus*, unreduced egg cells have been considered to be aposporic in origin, and it was assumed that embryos develop without fertilization. Development of embryos is, however, dependent on pollination which, presumably, is necessary for fertilization of the endosperm (unreduced pseudogamy). The results presented here show that the breeding behaviour of tetraploid *Rubus* species does not follow this accepted pattern, but that embryos develop from haploid embryo-sacs arising by a normal meiotic sequence from megaspore mother cells.

Three tetraploid species, *R. caesius*, *calvatus* and *laciniatus*, have been investigated and in all three species meiosis was normal and embryo-sac formation followed the *Polygonum* type of development. Development has been studied in particular detail in *R. caesius* since this species has been stated to be an obligate apomict[2]. All stages of meiosis have been found in the megaspore mother cell and at MI there were 14 bivalents. Subsequent chromosome separation was normal and a young tetrad was seen regularly. All stages in the development of the embryo-sac have been observed with the exception of the third embryo-sac division. There was no evidence of

Table 1. CHROMOSOME NUMBERS IN PROGENIES OF SEEDLINGS FROM *R. laciniatus*

	No. of seedlings								
	2n = 14	2n = 21	2n = 25	2n = 27	2n = 28	2n = 29	2n = 30	2n = 35	2n = 42
R. laciniatus ♀ ×									
2x* ♂		10	1		6		1	3	
2x† ♂		6			9			6	
4x ♂	1				13				1
Self	1			1	21	1			1
5x ♂					4				
6x ♂					2				
7x ♂					2				
	2	16	1	1	57	1	1	9	2

* × *R. ulmifolius*; † × *R. tomentosus*.

restitution and embryo-sacs, where counts could be made, had a haploid chromosome number. Thus there was no evidence of apospory.

Chromosome numbers of progeny from crosses between these three tetraploid species and other species of different ploidy have been determined. As an example of the variation found in chromosome numbers counts from crosses involving *R. laciniatus* are shown in Table 1. It will be seen that 62·3 per cent of the seedlings had the same chromosome number as the maternal species. The phenotypic appearance of these plants made it clear that they were identical to *R. laciniatus*. However, from the embryological evidence it is now known that they cannot be apomictic, since no unreduced embryo-sacs were formed.

In addition to these maternal-like seedlings there were both reduced and unreduced sexual seedlings, as shown by the plants having 21 and 35 chromosomes in families derived from diploid male parents. The former are considered to have been derived from fertilized reduced egg cells; the latter from reduced egg cells which, before fertilization, have become diploidized in the way described here. Plants with 21 chromosomes could be distinguished readily from those with 35 on phenotypic characters. The *laciniatus* leaf character is phenotypically recessive in the triploid and relatively more dominant in the pentaploid, where the proportion of *laciniatus* chromosomes is higher. The high frequency of plants with 35 chromosomes was unexpected and this class has not been reported previously. Relatively few seedlings with chromosome numbers different from *R. laciniatus* were formed in families derived from parents of higher ploidy. The two haploid seedlings with 14 chromosomes form a fourth class of progeny. These were derived directly from reduced egg cells. In addition, there were four aneuploids in the 91 seedlings examined.

The origins of two classes of progeny, the triploid sexuals and haploids, present no difficulty since the former are simply the products of reduced, fertilized egg cells while the latter have been developed from reduced unfertilized egg cells. Both classes have been reported previously and will not be considered further.

Since all megaspore mother cells in this material were seen to undergo meiosis, the production of tetraploid maternal-like and of pentaploid sexual progeny requires more careful consideration.

The former could arise in one of two ways: either restitution at the first division of the egg cell or 'fusion' of the nuclei within the embryo-sac, that is, automixis, must occur. Evidence of diploid parthenogenesis is found in *Petunia nyctaginiflora*[3]. This species, when pollinated with either *Salpiglossis variabilis* or *Nicotiana tabacum*, produces only homozygous *Petunia nyctaginiflora* seedlings. These are formed as a result of diploidization by restitution in the egg cell. Previous workers have suggested that automixis was a reproductive mechanism found in *Rubus*, although it has usually been considered to be of little general significance[4,5]. In the present material it was impossible to establish the method whereby doubling of haploid nuclei in the embryo-sac occurred. Since the genetic consequences of the two possible methods suggested above are the same, the critical establishment of the method of doubling is of minor significance.

Pentaploid sexual progeny must arise from egg cells which have undergone meiosis, become diploidized and later fertilized.

A further class of progeny which has been termed *sub-sexual* has been described by previous workers[6,7]. Phenotypically these are plants similar to the maternal species, have the same chromosome number, but are not genetically identical to it. Sub-sexual progeny are assumed to arise from diplosporic egg cells, which are themselves derived from megaspore mother cells in which a normal meiotic prophase is followed by restitution. Such egg cells have undergone genetic crossing over, but retain the somatic chromosome number. There is no cytological evidence for the occurrence of such a process in the tetraploid *Rubus* species studied.

One of the genetical implications of the reproductive mechanism now put forward is that the tetraploid species concerned are homozygous. However, since they are almost certainly amphi-diploids, as shown by their cytological behaviour during meiosis, there will be homozygosity only between the chromosomes of each of the two complexes which together make up the tetraploid. They will be heterozygous as a result of differences between the two complexes. Crossing-over, though it occurs, will not result in segregation, and diploidization either in the egg cell or as a result of automixis will immediately restore the full chromosome complement of the female parents. The sub-sexual plants might then arise from occasional allosyndetic pairing and crossing-over between the chromosomes of the two different complexes.

It has been shown before that the proportion of sexual progeny produced by a supposed apomict may differ with different pollinating parents. Further, it has been shown that species which in particular circumstances are sexual are, in different circumstances, also apomictic[8]. Thus *R. vitifolius*, when pollinated with diploid *R. idaeus*, gives only sexual progeny, whereas the same species when pollinated with the tetraploid form of *R. idaeus* gives both sexual and 'apomictic' seedlings. The breeding of this species is most easily explained on the basis that only one type of embryo-sac is formed, that is, sexual, and the subsequent development varies according to the pollinating parent used. If both sexual and apomictic seedlings were produced, it is difficult to understand why, in the cross involving diploid *R. idaeus*, apomictic progeny are not produced. These results also suggest that diploidization occurs at the first division of the egg, rather than as a result of automixis since, if the latter mechanism occurred, maternal-like seedlings would be expected from the cross using diploid *R. idaeus*.

Much of the evidence for apomixis in *Rubus* has been derived from chromosome counts[9]. The chromosome counts of apomictic seedlings will be identical

to those of the maternal species from which they arose. Many of the counts cited involve self and open pollinations. Since sexual seedlings derived from such crosses may also retain the maternal chromosome number, they are, however, inadmissible. Nevertheless, pollinations between parents of widely different ploidy do frequently produce a high proportion of maternal-like seedlings. In crosses between tetraploid female parents and various male parents, Einset found that the number of reduced parthenogenetic seedlings (43) was almost as great as the total number of sexual seedlings (46) and higher than might be expected when compared with the number of supposed apomictic tetraploids (592). These relative frequencies suggest that reduced embryo-sacs are of much greater significance than hitherto realized.

Two other recent accounts of reproductive mechanisms in *Rubus* are relevant to the present work. Christen found evidence in *R. caesius* that only sexual embryo-sacs are formed[10]. Similarly, Markarian and Olmo suggested that there was no evidence for the formation of aposporic embryo-sacs in another 'apomict', *R. procurus*[11]. It was assumed by these workers that the form of the species studied must have been atypical of the species generally, or that the aposporic stages of embryo-sac formation had been missed. Neither of these possibilities is true of the present work. Clones of *R. caesius* from England, Finland and Poland have now been examined and in all only reduced sexual egg cells were formed. Also, the frequency with which the sexual stages of development were seen makes it impossible to consider that the development of aposporic embryo-sacs has been overlooked.

The high proportion of seedlings with 35 chromosomes, which, as shown here, were derived from pollination of *R. laciniatus* with diploid male species gives still further evidence against an apomictic mechanism. Fertilization of aposporic egg cells would be unlikely to occur if apomixis, as is generally supposed, enables a species, which would become extinct under a sexual system, to reproduce itself.

The evidence not only from this study but also from previous work suggests, therefore, that apomixis in its commonly accepted sense, is not an important reproductive mechanism in tetraploid forms of *Rubus*. The production of maternal-like progeny, though superficially simulating apomixis, depends solely on the formation of reduced egg cells.

Much of this work was carried out at the John Innes Institute, and I am particularly grateful for the stimulating guidance of Mr. Watkin Williams and for the technical assistance of Miss P. Dow.

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SOME CHARACTERISTICS OF MITOCHONDRIA EXTRACTED FROM HIGHER PLANTS

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WITH the exception of one brief note[1] there are no instances in the literature showing control of respiratory activity in mitochondria isolated from higher plants. The purpose of this article is to show some of the characteristics of higher plant mitochondria that do possess such respiratory control.

There are instances on record (cf. ref. 2) which show a modest increase in plant mitochondrial oxidative activity (up to twice) in the presence of adenosine diphosphate. It is possible to obtain mitochondria which show no utilization of oxygen following addition of substrate (succinate) but which are maximally active following addition of a minute amount of adenosine diphosphate. The increase in rate of utilization of oxygen following addition of adenosine diphosphate is usually about ten times. In such instances (Fig. 1) the utilization of oxygen does not decrease on exhaustion of adenosine diphosphate, and there is a curious induction period following its addition before steady-state conditions are achieved. The induction period may well be related to the requirement for adenosine triphosphate for succinate oxidation (see discussion in ref. 3). The dependence of this type of plant mitochondria on adenosine diphosphate and the lack of control is not due to activity of mitochondrial adenosine triphosphatase but rather to several factors which sediment with starch and the mitochondria. The preparation of plant mitochondria that show oxidations controlled

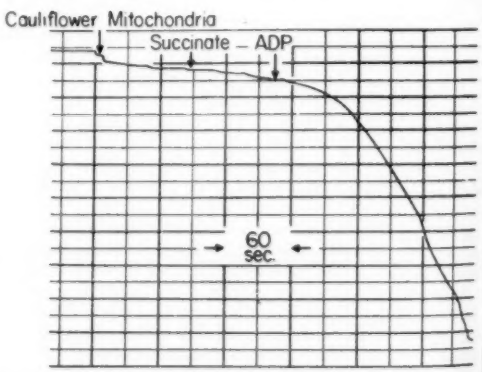

Fig. 1. Polarograph trace of the oxygen utilization by cauliflower mitochondria. Mitochondria suspended in a phosphorylating medium which consisted of: mannitol, 0·3 *M*; potassium chloride, 0·01 *M*; magnesium chloride, 0·005 *M*; potassium dihydrogen phosphate, 0·01 *M*; versene, 10^{-4} *M*; pH 7·4. Succinate = 0·005 *M*; the concentration of adenosine diphosphate resulting from each addition was 125 μM

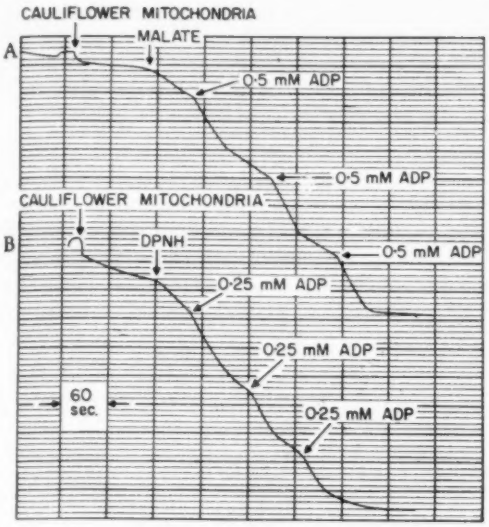

Fig. 2. Polarograph traces of oxygen utilization by cauliflower mitochondria in the presence of malate, trace *A*, and reduced diphosphopyridine nucleotide, trace *B*. Malate = 0·005 *M*, 2·5 mgm. reduced diphosphopyridine nucleotide added, conditions as in Fig. 1

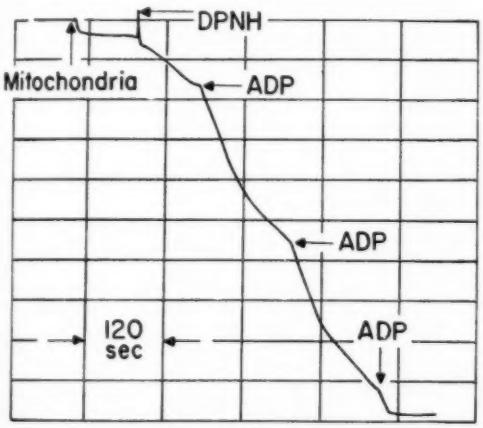

Fig. 3. Polarograph trace of the oxygen utilization by sweet potato mitochondria in the presence of reduced diphosphopyridine nucleotide and adenosine diphosphate. 2·5 mgm. reduced diphosphopyridine nucleotide added, conditions as in Fig. 1

by adenosine diphosphate depends on several factors, one of which is the careful separation of the mitochondria from the starch fraction.

Cauliflower (*Brassica oleracea*) and sweet potato (*Ipomea batatas*) have been shown to be excellent chlorophyll-free sources for mitochondrial investigations[1,2,4] and are easily obtained from local markets in some places.

Mitochondria were isolated in the following manner. 150 gm. of cold, diced tissue were ground in a Waring blendor for 5 sec. in 300 ml. of grinding medium. The grinding medium consisted of 0·6 *M* mannitol, 0·6 *M* sucrose (75 per cent mannitol, 25 per cent sucrose), 0·5 per cent cysteine (free base), and 10^{-3} *M* versene. The *p*H of the medium was 7·2. The partially disintegrated tissue resulting from the treatment in the Waring blendor was ground in small aliquots in an unglazed porcelain mortar, the grinding being continued until a smooth paste was obtained. The disintegrated tissue was squeezed through muslin and the resulting suspension was centrifuged at 1,000*g* for 5 min. The mitochondria were then sedimented by centrifuging 7 min. at 10,000*g*. The mitochondria were washed twice in the mannitol–sucrose mixture but without the addition of cysteine or versene. After each washing the sedimented mitochondria must be separated carefully from the starch fraction. The *p*H of all suspensions is one very critical factor in the preparation of mitochondria from cauliflower. With cauliflower, the *p*H must be kept between 7·2 and 7·5; with sweet potato, the *p*H is not such a critical factor.

Sweet potato and cauliflower mitochondria actively oxidize a few substrates. Fig. 2*A* shows the time-course of malate oxidation by cauliflower mitochondria, and it may be seen that the presence or absence of adenosine diphosphate considerably alters the rate of oxidation. In marked contrast to intact mitochondria from animal tissues, the plant mitochondria not only rapidly oxidize malate but also reduce diphosphopyridine nucleotide. The rate of oxidation of reduced diphosphopyridine nucleotide is also controlled by the presence or absence of adenosine diphosphate (Figs. 2*B* and 3). This control has been obtained in mitochondria derived from sweet

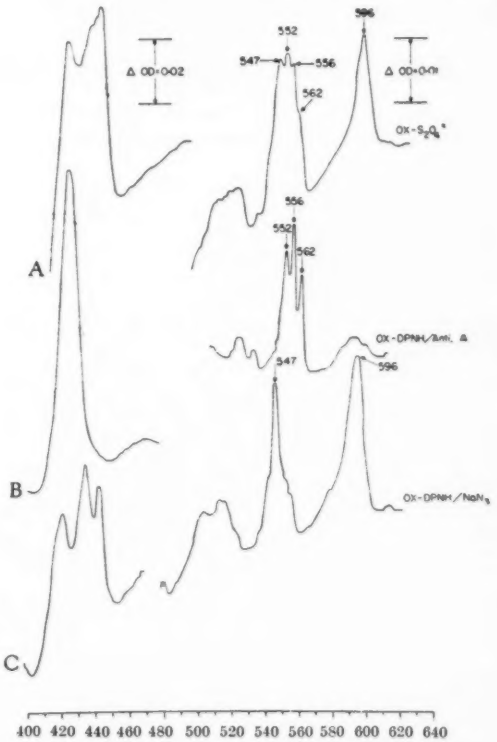

Fig. 4. Low-temperature (−190° C.) difference spectra of sweet potato mitochondria. Curve *A*, the difference in absorption between oxidized and hydrosulphite-reduced mitochrondria. Curve *B*, the difference in absorption between oxidized mitochondria and mitochondria reduced with reduced diphosphopyridine nucleotide in the presence of antimycin *A*. Curve *C*, the difference in absorption between oxidized mitochondria and mitochondria reduced with reduced diphosphopyridine nucleotide in the presence of azide

potato, potato (*Solanum tuberosum*), cauliflower, broccoli, beetroot and cucumber. It would seem that the oxidation of 'external' reduced diphosphopyridine nucleotide is a phenomenon common to plant tissues, and represents one of the marked differences between plant and animal mitochondria.

The cytochrome components of sweet potato mitochondria have been delineated through the use of a sensitive rapid scanning split-beam spectrophotometer[5] adapted for use at low temperature[6]. The spectra were obtained in the absence of glycerol as described by Bonner[7]. Low-temperature spectrophotometry allows increased absorption and increased delineation, afforded by the sharpening of absorption bands at such temperatures[8]. A low-temperature difference spectrum between aerobic and hydrosulphite-reduced sweet potato mitochondrial suspensions shows maxima at 596, 562, 556, 552 and 547 mμ (Fig. 4*A*). A similar spectrum of the difference in absorption between aerobic mitochondria and mitochondria reduced with reduced diphosphopyridine nucleotide in the presence of antimycin *A* shows maxima at 562, 556 and 552 mμ (Fig. 4*B*). Finally, a difference spectrum between aerobic mitochondria and mitochondria reduced with reduced diphosphopyridine nucleotide in the presence of azide shows two main peaks at 596 and 547 mμ (Fig. 4*C*). These difference spectra show maximum absorption at 596 and 547 mμ, corresponding to the maxima of cytochromes $a + a_3$ and *c* respectively. Since the maxima at 562, 556 and 552 appear in the spectrum obtained in the presence of antimycin *A*, they are interpreted to represent the absorption maxima of three different *b* cytochromes.

The cytochromes of sweet potato mitochondria appear to be identical to those which have been described in mitochondria derived from a variety of plant tissues[7]. The cytochromes present in higher plant mitochondria are remarkably similar among plant species, but show marked differences from animal mitochondria, especially in the nature of the *b* components. It is our hope that the preparation of reasonably intact mitochondria, as described here, will provide the much-needed material for the characterization of the respiratory chain in plants.

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GIBBERELLIN-LIKE SUBSTANCES IN PLANTS

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Leguminous Root Nodules

It has been shown[1] that in clover excision of the first-formed nodules, and also of the root-tips, stimulates further nodulation. Plants grown in association[2] or in succession[3] on a limited volume of medium can inhibit nodulation of an inoculated leguminous plant. The donor of the inhibiting effect need not be a leguminous plant. When charcoal[4] is added to the rooting medium, nodulation-rate is stimulated. It has also been shown that root-nodule formation on leguminous plants is reduced in *Phaseolus*[5,6] and in some species of *Trifolium*[7] by treating the foliage with gibberellin.

The content of endogenous gibberellin-like substances in nodules and roots has now been examined by the bioassay of extracts of these tissues. The extractions were carried out with ethyl alcohol, and the solutions were purified as previously described[8]. The extracts were then applied in alcohol to the leaves of dwarf pea seedings (var. Meteor). The growth of these plants was compared with others treated with known amounts of gibberellic acid.

Table 1 gives the results of three experiments using French beans and garden peas. The first experiment was carried out with young bean seedlings, one month old. In the second experiment old plants, from which pods had been gathered, were used. In neither case was any gibberellin-like activity detected in the roots, whereas a high concentration was found in the nodules. Dwarf and tall varieties of pea were compared. In both cases a low concentration of active substances was detected in the roots and a high concentration in the nodules. The nodules of the dwarf variety appeared to be particularly rich in these substances.

In addition, two small-scale experiments were carried out with French bean plants grown in water culture with and without inoculation with *Rhizobium*. In one experiment the nodulated roots and the culture solution in which they were grown both contained material with gibberellin-like activity, whereas none was detected in the uninoculated sample. In the other experiment the same amount of activity was found in the shoots of both groups of plants and some activity in the nodules of the inoculated plants, but none was found in the roots or the culture solutions of either group.

No activity was detected in a 2-gm. sample of root tips from 3-week-old French beans grown in soil;

Table 1. Gibberellin-like Activity in Roots and Nodules

Species	Tissue extracted (gm.)		μgm. gibberellic acid equivalent per kgm. tissue
I. *Phaseolus vulgaris* var. Masterpiece	Nodules	8·2	9·8
	Roots	8·2	0
	,,	24·9	0
II. *Phaseolus vulgaris* var. Masterpiece	Nodules	8·2	17·1
	,,	16·8	26·2
	Roots	8·2	0
	,,	108·5	0
III. *Pisum saticum* var. Meteor	Nodules	0·3	240
	Roots	0·3	0
	,,	69·3	0·2
var. Improved Pilot	Nodules	0·26	35·4
	Roots	0·26	0
	,,	59·7	0·93

but French bean seedlings grown in water culture secreted material with gibberellin-like activity into the culture solution for at least two weeks after germination. At least 0·01 μgm. gibberellic acid equivalent per plant was detected. Runner beans, peas, wheat and radish secreted smaller quantities into the culture medium.

These results suggest that the nodule-inhibiting factor produced by nodules and root tips is probably a gibberellin, and this may diffuse out into the surrounding medium in some circumstances.

The plants inoculated with *Rhizobium* in water culture were provided by Mr. W. K. Smith.

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Seaweed (*Fucus vesiculosus*)

ALTHOUGH gibberellin-like substances have been found in many species of flowering plants, none has yet been reported from lower plants, apart from the fungus *Gibberella fujikuroi* and the yeast *Candida pulcherrima*[1]. Nevertheless, there are reports of a response to applied gibberellin in ferns[2,3], a moss[4], a liverwort[5] and green algae[6,7], suggesting that gibberellins may play some part in regulation of growth. A substance with gibberellin-like activity has now been shown to occur in a seaweed.

4,000 gm. of *Fucus vesiculosus* was collected and stored at − 18° C. For the first experiment 500 gm. was extracted with ethyl alcohol for 18 hr. at room temperature. The alcohol was removed by filtration and pressing, and the tissues were re-extracted with fresh 70 per cent aqueous alcohol for 4 hr. The alcoholic solutions were combined and were then treated as previously described[8]. The purified extracts were applied in alcohol to the leaves of dwarf pea seedlings of the variety Meteor. Three doses of the extract were compared with known amounts of gibberellic acid. This test was carried out in duplicate, one batch of plants being grown in a greenhouse in the natural lighting conditions of July, and the other batch grown under continuous artificial light only at a temperature of 15° C. The results are given in Table 1.

Table 1. GIBBERELLIC ACID EQUIVALENT (μGM./KGM. FRESH WEIGHT)

Fresh weight equiv. of extract (gm.) per plant	Bioassay conditions	
	Greenhouse	Artificial light
50	7·2	0·76
10	12·0	2·1
2·5	6·8	5·2

Thus all extracts promoted growth considerably; this is evidence for the presence of gibberellin-like substances. The relative decrease in activity in the higher doses tested, when the plants were receiving artificial illumination, suggested that inhibitors might be interfering with the response.

The remaining 3,500 gm. of material was then extracted twice with alcohol, filtering but not pressing the tissue each time, and the alcoholic solution was concentrated in a climbing film evaporator. The acidic fraction of this was adsorbed on to a column of charcoal/'Celite' (5 : 1), and was eluted with water containing increasing amounts of acetone. Six fractions were recovered from the aqueous acetone and were dissolved in ethyl alcohol.

In a preliminary series of bioassays aliquots of all eluates were concentrated and applied to dwarf pea plants. Gibberellin-like activity was found only in the 50 per cent acetone eluate. In a similar experiment carried out under artificial light in which 1·0 μgm. gibberellic acid was added to each eluate it was found that the 90 per cent acetone eluate (an aliquot equivalent to 100 gm. of tissue) reduced the response to gibberellic acid to about half that expected; there was no evidence for the presence of an inhibitor in the 50 per cent acetone eluate.

The 50 per cent acetone eluate was then bioassayed at three dosage-levels (Table 2). The lower level of activity found, compared with that recorded in Table 1, may have been due to loss in storage or to less-efficient extraction. Part of this eluate was chromatographed on paper in *n*-butanol/1·5 *N* ammonia (3 : 1). The paper was divided into 10 parts, eluted with alcohol and bioassayed on dwarf peas. All the activity was found between R_F 0·3 and 0·4. Under these conditions the R_F of gibberellic acid was 0·38 and that of gibberellin A_5 was 0·59. The other known gibberellins with a similar R_F to gibberellic acid are gibberellins A_1 and A_6 (ref. 9); thus the active material present in *Fucus vesiculosus* may be one of these.

Table 2. GIBBERELLIC ACID EQUIVALENT (μGM./KGM. FRESH WEIGHT)

Fresh weight equiv. of extract (gm.) per plant	Bioassay conditions	
	Greenhouse	Artificial light
125	0·45	0·36
25	0·52	0·40
4	trace	trace

Chromatograms of the 90 per cent acetone eluate were run in the same solvent system. An inhibitory substance present at R_F 0·6–0·7 was demonstrated by a lettuce germination test and by the lettuce seedling assay[10] in which the response to gibberellic acid was reduced. On the other hand, there was no significant reduction of the response of dwarf peas to gibberellic acid, and it is not clear whether this is in fact the substance causing the reduction in gibberellin response in the earlier experiments. Inhibitor β, which is found in many higher-plant tissues, has a similar R_F in this solvent system[11,12]. An inhibitor of gibberellin-induced growth found in seed extracts has been described by Corcoran and Phinney[13]. Its mobility in this solvent system is not known.

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AN ENDOGENOUS RHYTHM IN THE RATE OF DARK-FIXATION OF CARBON DIOXIDE IN LEAVES OF *BRYOPHYLLUM FEDTSCHENKOI*

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LEAVES excised from the succulent plant *Bryophyllum fedtschenkoi* and maintained in darkness, at a constant temperature of 26° C., and in an air-stream initially free of carbon dioxide, exhibit an endogenous circadian rhythm in their rate of carbon dioxide emission[1,2]. The rhythm is inhibited by light but recommences when darkness is restored, the phase being determined by the time at which the light is extinguished. Furthermore, exposing leaves maintained in darkness to a light perturbation of 5 hr. duration at the crest of a peak has no effect on the phase, whereas a similar perturbation offered between the peaks induces a phase shift in which the first post-treatment peak occurs 18–19 hr. after the recommencement of darkness. The rhythm of emission of carbon dioxide is present in leaves excised from plants grown during long days and short days[1].

It has been suggested[1] that this rhythm may be due to the periodic activity of a dark, carbon dioxide fixation mechanism acting on an aperiodic rate of production of respiratory carbon dioxide by the tissue and the present work was designed to test this hypothesis.

The experimental plants were grown in cabinets in which illumination was provided by a Philips high-pressure mercury vapour lamp and four 150-W. tungsten lamps. Long-day illumination extended from 2400 hr. until 1600 hr. and short-day illumination from 0800 hr. until 1600 hr. The technique adopted in these experiments was as follows: three leaves were placed in each of a number of specially constructed glass tubes through which an air stream, initially free of carbon dioxide, passed at a rate of 1·5 l. per hr. The tubes were immersed in a water bath at 26° C. and illuminated at an intensity of 3,000 lux until darkness commenced. At 2-hr. intervals following the onset of continuous darkness, the flow of air free of carbon dioxide to one of the tubes was interrupted and $^{14}CO_2$ liberated in the closed tube. The concentration of carbon dioxide in the tube at the commencement of the 2-hr. feeding period was 0·66 per cent and the specific activity 88·4 μc./m.mole. At the end of the feeding period the tube was cleared of labelled carbon dioxide and the leaves were killed in boiling 80 per cent alcohol. They were then macerated and extracted successively with 80 per cent alcohol, 50 per cent alcohol and water, when no activity remained in the solid residue. The extract was reduced to a known small volume and the total carbon dioxide fixed by the leaves during the feeding period determined by estimating the radioactivity present in a sample of the extract. The uptake of carbon dioxide is expressed as μgm. carbon dioxide per hr. per gm. fresh weight of tissue. Simultaneous control experiments were conducted in which the rate of carbon dioxide output of two samples of leaves was monitored with an infra-red gas-analyser incorporated in an apparatus similar to that used in previous studies[1–3].

In the first two experiments leaves were excised from the plants between 1400 hr. and 1500 hr. and transferred to continuous darkness at 1600 hr., that

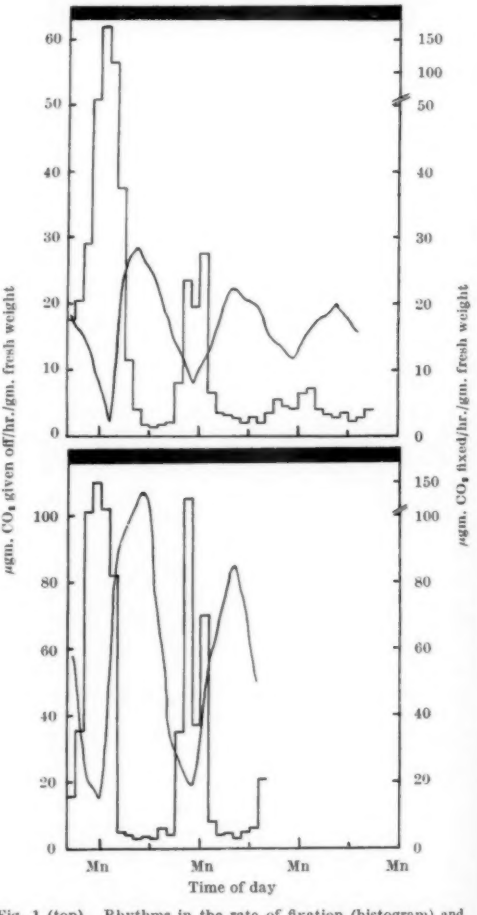
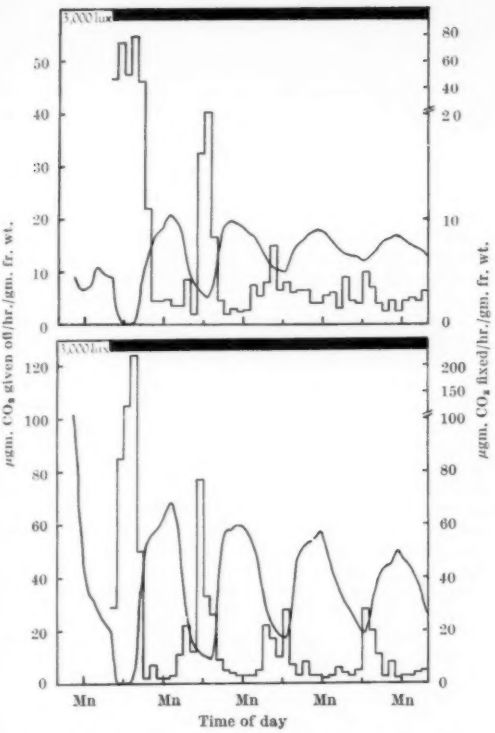


Fig. 1 (top). Rhythms in the rate of fixation (histogram) and output (continuous curve) of carbon dioxide in excised short-day leaves of *B. fedtschenkoi* maintained at 26° C. and in continuous darkness which commenced at 1600 hr. The black area of the bar above each figure indicates darkness. Ordinates, μgm. CO_2 fixed or given off/hr/gm. fresh weight of tissue; abscissa, time of day, Mn (midnight)

Fig. 2 (bottom). Rhythms of fixation (histogram) and output (continuous curve) of carbon dioxide in leaves taken from a plant grown in long days and transferred to darkness at 1600 hr.

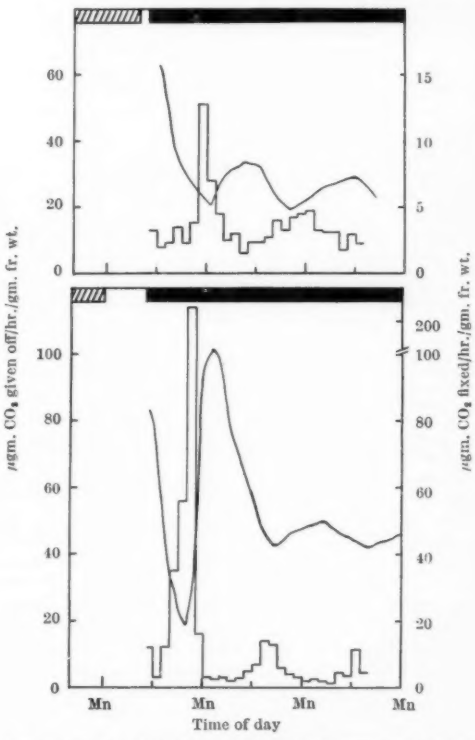


Figs. 3 and 4. Changes induced in the phase of the rhythms of fixation (histogram) and output (continuous curve) of carbon dioxide in short-day (Fig. 3, above) and long-day (Fig. 4, below) leaves by postponing the onset of continuous darkness from 1600 hr. to 0800 hr. the following morning

is, at the same time that darkness commences in the growth cabinets. The subsequent rate of dark fixation of carbon dioxide by short-day leaves showed a distinct rhythm in which the first peak occurred at 2400–0200 hr., 8–10 hr. after the onset of continuous darkness, and further peaks at intervals of approximately 23–24 hr. (Fig. 1). A similar result was obtained when this experiment was repeated with leaves taken from a plant grown in long days (Fig. 2). Continuous darkness again commenced at 1600 hr. and the first peak of the rhythm of fixation occurred approximately 8 hr. later at 2400 hr., with subsequent ones at about 23-hr. intervals. In each experiment the peaks of the rhythm of fixation occurred at times closely similar to the troughs in the rhythm of net output of carbon dioxide and vice versa. The slightly earlier occurrence of the first peak of the fixation rhythm in long-day leaves is consistent with a previous finding[1,4] that the transient in the output rhythm decreases as the duration of the previous light treatment is increased.

The phase of the rhythm of fixation of carbon dioxide can be changed if the onset of darkness, which normally occurs at 1600 hr., is postponed for several hours. Leaves were excised and placed in the tubes in the usual manner between 1400 hr. and 1500 hr. and then illuminated at an intensity of 3,000 lux until 0800 hr. the following morning. The rate of carbon dioxide fixation by both short-day (Fig. 3) and long-day (Fig. 4) leaves in the subsequent continuous darkness showed a distinct 23–24-hr. rhythm in which the first peak occurred at approximately 1400 hr., that is, about 6 hr. after the light was extinguished. Figs. 3 and 4 show that the rhythm of carbon dioxide fixation persists for at least 4 days in both short-day and long-day leaves held in darkness and at 26° C. In each figure the peaks of the fixation rhythm again occur at similar times to the troughs in the output rhythm.

In two further experiments the leaves were excised between 0800 hr. and 0900 hr. and transferred to darkness at 1000 hr.; both the short-day and the long-day leaves therefore experienced darkness 6 hr. earlier than if they had remained in the growth cabinets. It is clear from Fig. 5 that when short-day leaves are darkened at 1000 hr. the first peak of the rhythm of carbon dioxide fixation occurs at 2400–0100 hr. and the next peak 22–23 hr. later. The peaks occur, in fact, at the same times of day as those in the experiment shown in Fig. 1, where the leaves were darkened at 1600 hr. On the other hand, if long-day leaves are darkened at 1000 hr. the first peak of the fixation rhythm occurs at approximately 1900 hr., 9 hr. after the onset of darkness (Fig. 6), and subsequent ones at intervals of approximately 23 hr. It is clear, therefore, that while the early onset of darkness does not modify the phase of the rhythms of fixation or output of carbon dioxide in short-day leaves, in long-day leaves



Figs. 5 and 6. Rhythms of fixation (histogram) and output (continuous curve) of carbon dioxide in short-day (Fig. 5, above) and long-day (Fig. 6, below) leaves transferred to continuous darkness early in the day at 1000 hr. The hatched area of the illumination programme bar represents the darkness experienced by the leaves in the growth cabinets prior to the onset of the day (unshaded area) in which they were excised and darkened early. For explanation see text

a phase shift is induced in which the first peaks of the fixation and output rhythms occur approximately 9 and 19 hr. respectively after the recommencement of darkness.

The invariable coincidence between the times of occurrence of the peaks of the fixation rhythm and the troughs of the output rhythm and vice versa in Figs. 1–6, irrespective of the time of day at which darkness commences, indicates that the rhythm of emission of carbon dioxide in the leaves is, in fact, a manifestation of the fixation rhythm re-fixing respiratory carbon dioxide at certain times in the cycle and not at others.

The induction of a phase-shift in the rhythms of both fixation and output of carbon dioxide by exposing long-day, but not short-day, leaves to darkness at 1000 hr. was to be expected on the basis of results already published[2,3]. If the onset of the dark period immediately prior to the day during which the leaves were transferred to darkness at 1000 hr. is considered as the time when continuous darkness commenced, then the curtailed day which the leaves received afterwards can be regarded as a light perturbation. In the case of long-day leaves this perturbation commenced at 2400 hr., that is, between the peaks of the rhythm of carbon dioxide output, and this would induce a phase-shift in which the first post-treatment peak would occur 18–20 hr. after the end of the treatment. In the short-day leaves the perturbation did not commence until 0800 hr., that is, until the crest of the peak of the output rhythm had been reached, and the phase of the rhythm is unaffected by a perturbation offered at this position in the cycle. Furthermore, the duration of the perturbation experienced by the long-day leaves was 10 hr., whereas that experienced by short-day leaves was only 2 hr.

Except when the leaves are, in effect, merely exposed to a light perturbation at the crest of a peak of the output rhythm, it is clear that the phase of the rhythms of output and fixation of carbon dioxide is determined by the time at which darkness commences and may be set accordingly at any desired time of day. Moreover, the results presented in Figs. 5 and 6, and those already published[2,3], show that the phase of the rhythms in leaves maintained in darkness is reset by a light perturbation only if it is administered in that part of the cycle where carbon dioxide fixation is occurring.

In attempting to locate the basic, oscillating chemical system in the leaf tissue it is important to determine whether the rhythm of carbon dioxide fixation is the only component of the output rhythm or whether there is also a rhythm in the rate of production of respiratory carbon dioxide. Preliminary experiments using the Warburg technique have not hitherto yielded evidence of a rhythm in the rate of respiration (oxygen uptake) of either whole or dissected leaves maintained in darkness and at 26° C. If the rate of production of respiratory carbon dioxide is aperiodic and corresponds to the rate of carbon dioxide emission of the leaves at the crests of the peaks of the output rhythm, then there should be a quantitative agreement between the maximum rates of fixation of carbon dioxide and the maximum decreases in the rates of carbon dioxide emission by the leaves in the corresponding troughs of the output rhythm. However, it is not possible to make a close quantitative comparison between the maximum rates of carbon dioxide fixation and the maximum decreases observed in the rate of carbon dioxide output of the leaves because, first, the rate of fixation of carbon dioxide by the leaves is a function of the ambient concentration of the gas, and secondly, the rates of fixation of carbon dioxide recorded in this work refer to the total fixation of the carbon dioxide liberated into the tubes and do not include the refixation of respiratory carbon dioxide which must occur during the 2-hr. feeding period when the leaves are incorporating the labelled gas.

The endogenous rhythm in the rate of dark fixation of carbon dioxide is undoubtedly the major component of the rhythm of carbon dioxide output in the leaves. Whether or not it is the only component is now the subject of a detailed investigation.

We thank Prof. T. A. Bennet-Clark for providing laboratory facilities for this work, and the Agricultural Research Council for financially supporting one of us (D. M. W.).

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CALCULATION OF THE AREAS OF ISOLATED OR OVERLAPPING NORMAL PROBABILITY CURVES

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A MATHEMATICAL method has been developed for the calculation of the areas of normal probability curves, even when they overlap one another[1]. The method was used in that paper for the analysis of gas chromatograms obtained with a differential detector system. It should be possible to apply this method to any display of overlapping curves which exhibit probability characteristics. There are many procedures in various fields which apparently give such displays besides gas chromatography; for example, there are the curves obtained on plotting a given chemical property versus emergence time or fraction number in column partition chromatography; the optical density curves obtained by photoelectric scanning either through an angle in X-ray spectrometry or over a wave-length in emission spectra or across the spots in the direction of their motion in paper chromatography or electrophoresis; the sedimentation diagrams obtained on the ultracentrifuge with materials of high molecular weight; and some mass spectrometry curves, particularly those obtained on machines using the time of flight principle. The optical density curves obtained from photographic plates of emission spectra or Debye–Scherrer X-ray

diffractions may also be probabilistic in nature, but calculations for such curves will depend on variations in the photographic response with wave-length and intensity. There is also the possibility that this method might apply to some very simple, uncomplicated infra-red or ultra-violet spectra.

The first step in the application of this method is to determine whether isolated peaks fit the requirements for normal probability curves. From Sheppard's tables of the normal probability integral[2], it is found that the width of the normal probability curve equals one standard deviation, σ (sigma), at 0·882 of the peak height, h, equals 2σ at 0·607 h and 3σ at 0·324 h. If the curve conforms to a normal probability curve, the values of σ derived from measurements of peak-width will be constant. The area may then be calculated from the equation: area, $A = h\sigma\sqrt{(2\pi)}$.

For the determination of the areas of overlapping normal probability peaks, there must be some means of predicting the σ values these peaks would have if they were isolated. In the case of chromatographic processes, the distribution of a given compound is controlled by random mechanisms such as diffusion within a phase or transfer between phases. The repeated application of these random mechanisms leads to broadening the peaks (increased σ) as the process continues. In gas chromatography, when σ is plotted versus the retention times of the peaks, a straight line is obtained[1]. The work of Craig[3] indicates that the same increase in σ occurs in countercurrent distribution and in column chromatography. For other processes, σ may be constant, or depend on certain experimental variables, for example, width of slit in an optical system or angle from the incident beam, in a diffraction system. All the above variations of σ with time, fraction number, angle, slit width, etc., are experimental variables which can be correlated to values of σ in a calibration curve. Sigma can then be read from this curve and the σ values of overlapping peaks can be interpolated.

When two additive normal probability curves overlap, the contribution of one curve to the apparent height of the other can be calculated. The information necessary for this correction was obtained from Sheppard's tables[2]. For convenience, it is presented graphically on probability paper in Fig. 1 with the ordinate of the normal probability curve, converted to percentage of peak height, plotted against the distance between the peaks measured in units of σ.

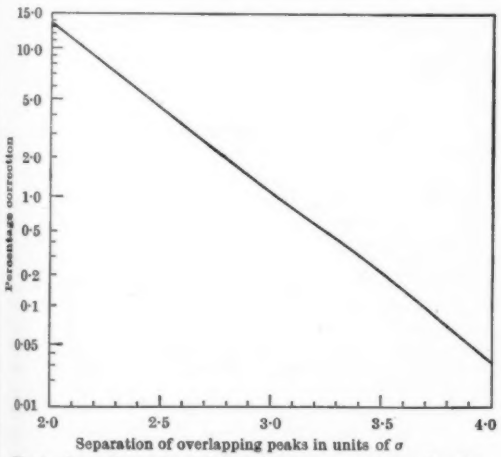

Fig. 1. Corrections of peak height for given separations of peaks measured in units of σ of the interfering peak

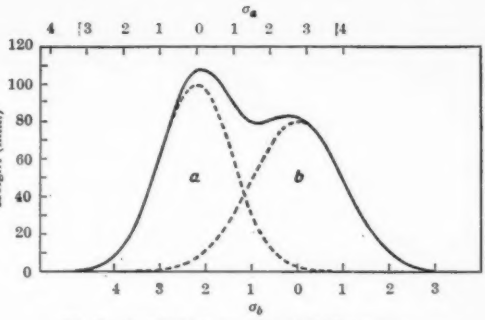

Fig. 2. Overlapping normal probability curves

A sample calculation of the mutual correction of two peaks is as follows. Consider the two peaks in the construction in Fig. 2. From previous experiments carried out under the same experimental conditions, either on the two isolated components for most accurate work, or on various mixtures, the values of σ and positions of the two peaks are interpolated. In the example under consideration, the values of σ obtained from a calibration graph are, for the first peak, (*a*) 20 mm. and for the second peak, (*b*) 25 mm.; the peaks are 55 mm. apart before photographic reduction for publication. The contribution of peak *b* to the height of peak *a* at the mean of peak *a* is dependent on the distance *a* is from *b* in terms of σ_b, which is 55/25 or 2·2 σ_b. This corresponds to a percentage contribution of 8·8 per cent of 82 mm., the observed height at *b*, that is, 7·2 mm. The observed height at *a*, 107 mm., is then corrected to 99·8 mm. by the deduction of the contribution of peak *b*. This figure is then used in the calculation of the true area of peak *a*. Similarly, the contribution of peak *a* to the height of peak *b* at its mean depends on the distance apart of *a* and *b* in terms of σ_a, which is 55/20 or 2·75 σ_a, corresponding to a contribution of 2·2 per cent of the 99·8 mm. of peak *a* or 2·2 mm. to be deducted from the height of peak *b*, giving 79·8 mm. for the true height of peak *b*. The construction actually used a height of 79·8 mm. for *a* and 99·7 mm. for *b*, giving an accuracy for the method within the limits of linear measurement on such a construction.

This accuracy was obtained with what is only the the first of a series of iterative corrections. It has also been observed that when the peaks are 4σ or more apart, no corrections need be made in the measured peak height.

It can be seen from Fig. 1 that there is a slight horizontal displacement of the observed peaks when they approach as closely as 2σ. The effect of this is insignificant; in fact, in many cases, if the peaks are separated enough to show as defined shoulders, direct measurement of height will give values by calculation for the areas which are within the inherent precision of the method itself.

This method may be extended to more than two peaks with the appropriate interrelated series of multiple corrections. Even with skewed probability curves, a similar treatment can be used to find the area of overlapping peaks[1].

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LETTERS TO THE EDITORS

PHYSICS

Photography of Čerenkov Light from Extensive Air Showers in the Atmosphere

Several authors[1-4] have shown that Čerenkov light from extensive air showers in the atmosphere can be detected above the background light of the night sky, and that a single photomultiplier can be used for detecting showers of about 10^{15}-eV. primary energy at distances up to 600 m. from the shower axis.

We have used an image intensifier system, triggered by amplified pulses from a 5-in. photomultiplier, in an attempt to photograph the showers. The system consists of a Schmidt mirror 30 cm. in diameter, and nominal aperture of *f*0·5, but with usable area only 300 cm.^{2}. It has an acceptance cone of half-angle 17° about the zenith and is focused for infinity on the 5-in. diameter cathode of an image intensifier (Westinghouse *WX*4171). This is run continuously and integrates night-sky light over the decay time of the *P*15 phosphor, nominally 1 μsec. It is coupled optically to a three-stage cascaded intensifier (R.C.A. *C*73491) which is normally gated off, and which is followed by an intensifier orthicon, with kinescope display. The intensifier system has previously been used with a scintillation chamber and is described elsewhere[5]. Under present conditions we are limited by night-sky background, and operate the system well below full gain.

Light pulses are accepted by the photomultiplier over a cone of half-angle 50°, so that selection is isotropic over the field of view of the Schmidt system, but not all selected showers will be visible to it. Operation has been possible for 8 hr. under clear sky conditions and 32 shower pulses have been observed. A random pulse is applied a few seconds after each Čerenkov pulse, to obtain a comparison picture. Ten of the Čerenkov photographs have large spots, 3–5° in angular diameter. A typical event is shown in Fig. 1. No spots of comparable size or brightness have been observed in any of the 32 random pictures, though stars appear in both Čerenkov and random pictures. On covering the system, the field becomes dark. The evidence indicates that we are photographing the Čerenkov radiation from extensive air showers.

In the case illustrated, the first-magnitude star α Lyræ (Vega) also appears in both exposures, and this can be used to set a lower limit for the brightness of the shower pulse. It is known from previous work with the system that the effective integration time of the first phosphor is approximately 10 μsec., about ten times the nominal decay-time of the fast component. The total visible flux from the star during this time is roughly 10 photons/cm.^{2}. From the film density we estimate that the intensity of the shower pulse is of the order of 50 photons/cm.^{2}, consistent with a shower size of 10^5 particles. Accurate estimates are difficult because of non-linearities in the kinescope display and film response.

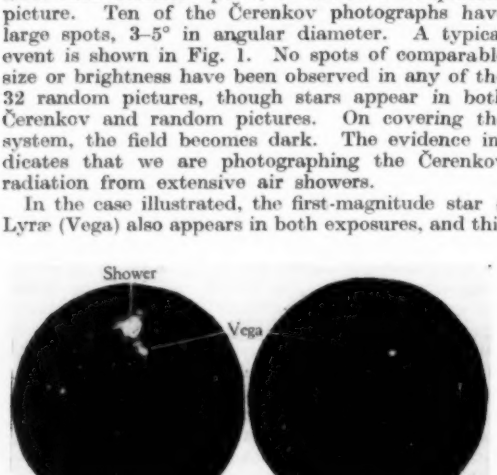

Fig. 1

The centres of the shower spots, which correspond presumably to the directions of the showers, can be determined with accuracy better than 0·5°. This combination of high directional precision with relatively high detection-rate suggests that the technique may be of value in the search for showers initiated by primary γ-rays.

The work was carried out at the Agassiz Observatory of Harvard University (100 m. above sea-level) and we would like to thank the Committee of the Observatory for allowing us the use of an astronomical building as well as Mr. K. Richards for his help in constructing and operating the system. One of us (N. A. P.) thanks the O.E.E.C. for the award of a senior visiting fellowship at the Massachusetts Institute of Technology. The experiment was supported in part through A.E.C. Contract *AT*(30-1)—2098, by funds provided by the U.S. Atomic Energy Commission, the Office of Naval Research, and the Air Force Office of Scientific Research.

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An Autoradiographic Effect

While using X-ray films in another investigation, autoradiographic effects were obtained from many ordinary substances or mixtures of them. Materials such as zinc metal powder alone or mixtures of metal powders in a medium such as dried 'Glyptal' varnish were used. Such mixtures were put in holes in wooden or plastic blocks and the films attached to the blocks with drawing-pins or tape. A cardboard separator was used around the edges of the blocks to keep the films out of contact with the materials, leaving an air-gap of about 1 mm. Exposures of 48 hr. were generally used.

The combination of materials used in most of these experiments was a mixture of aluminium filings in 'Glyptal' varnish with addition of small amounts of castor oil sometimes. The following experimental

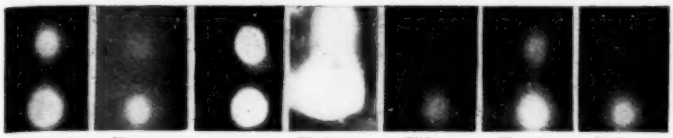

Fig. 1

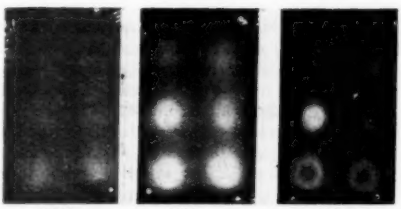

Fig. 2

results are for such mixtures, but similar results were obtained from mixtures of other materials and also from metal filings or powders alone, notably zinc. Metal powders are not necessary for the effects, for they were also obtained from mixtures of organic materials.

The following series of experiments were performed to find out their effect on the autoradiations that were obtained:

Effect of ambient atmosphere on the radiation: Blocks were made up and exposures made in different atmospheres. The block, in each case with film attached, was put in a box impervious to light and placed in a bell jar in which the atmosphere was changed for each exposure. Results are shown in Fig. 1. All exposures were from the same block and were for 48 hr. The pressure in each case was a full atmosphere, except for vacuum, which was about 25μ or what is obtained from a rotary oil vacuum pump. There was no 'out-gassing' of the materials. Gases used such as argon, helium and krypton were taken from supplies used for lamp making and were dry. Hydrogen was dried with a small tube containing phosphorus pentoxide.

It may be noted that the kind of atmosphere affects greatly the kind of radiation results obtained from the same materials. The intensity is changed, and in some cases the wood of the block is made to radiate as shown in the case for vacuum. The highest intensity was obtained for hydrogen. The effect of additions is shown here, because the lower spot was from the mixture with a few per cent castor oil, while the upper one had no castor oil.

Effect of ambient temperature: The effect of ambient temperature is shown in Fig. 2. The same block was used for all cases. It may be noted that ambient temperature changes the results as to intensity of radiation and also the areas of the block which produce the radiation. At the first two temperatures the spot radiates as a whole, but at the third the radiation comes mostly from the edges of the spot.

Transfer of radiation: This is an effect which was found to occur if exposed, but not developed, films were stored next to each other for as much as 24 hr. This effect was studied by itself, and the results are shown in Fig. 3. On the left side are original radiations from the blocks, but on the right side are results from films which were not exposed to blocks, but were left in contact with the films on the left before they were developed. The time of such contact was 24 hr. or more. As shown, definite transfers were made and it should be noted that the transfers are not in proportion to the intensity of the original films. This would indicate that the agency causing the transfer is not the same as that which affects the X-ray film. Such transfers were obtained from materials other than films exposed to the radiating blocks, such as sheets of gelatine and metal foil.

Attempts were made to produce such transfers from films exposed to known radiations such as ordinary light, X-rays (about 1·5 Å.), β-rays from tungsten-185, γ- and β-rays from iron-59 and infra-red light. In all cases the original films (except the infra-red) had images comparable with those obtained from the blocks, but no transfers were obtained. The film is not sensitive to infra-red.

Experiments were performed to find out how long the originally exposed film would produce such transfers. A film was exposed to a radiating block for 48 hr. It was then left in contact with various other films which were changed at 24-hr. intervals. The results are shown in Fig. 4. The original film is on the left and the others are the films that were in contact with the original for the first 24 hr., second 24 hr., third 24 hr. respectively. The third film did not reproduce, but the images were discernible in the negative. Thus the activity of the original film persisted for 72 hr. or more.

It should also be stated that, for the transfer, the two films have to be as close as 1 or 2 mm. between them.

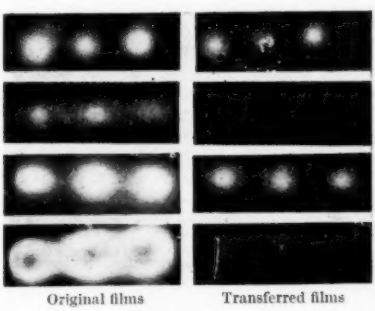

Fig. 3

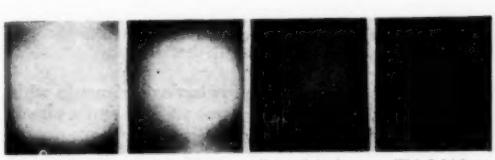

Fig. 4

Range of radiation: This, of course, depends on the materials involved. Exposures at a distance of 3 in. produced the effect on the X-ray film. Using the usual extrapolation techniques, the indication is that the radiation may extend to as much as 6 in. Also at 3 in., the same results were obtained with the radiating materials above, below or to one side of the films. It is considered that this would not occur if the effect were due to a gas or vapour.

Also, the effect on the X-ray film has been obtained through thin sheets of aluminium foil, mica and up to 0·5 mil. 'Mylar' sheet. Whether the actual original radiation goes through these materials is open to question, for it may be another case of transfer as described here, the intervening materials re-radiating to the film on the other side.

Life of the radiating bodies: Experimental results indicate that, so long as the materials remain in the same condition, they will continue to radiate in the same way. Since, however, some oxidize, others such as plastic materials polymerize and set, etc., their radiating characteristics change.

All films referred to as X-ray films were Eastman 'No screen' X-ray films. A few attempts were made to use other films such as 'TriX' and 'Panatomic', but without success.

The literature as listed here[1-3] mentions the 'Russell effect', which ascribes the effect of various materials on photographic films to the production of hydrogen peroxide, which reacts chemically with the films to produce the effect. In those cases ordinary films were used, and there is no doubt that hydrogen peroxide affects the films. In the above work, the effects were obtained only on X-ray film and were obtained under conditions such as vacuum, inert atmosphere and time where no such peroxide would be formed. This would indicate the presence of a radiation of a type for which these X-ray films are designed. It is therefore suggested that these radiations are a soft electron or β-type radiation with a range of as much as 6 in. from the source. The agency producing such radiations is somewhat obscure. It would have to be something which depends on the kinetic condition of the source, since production of the radiations is varied by conditions such as atmospheric content and pressure, vacuum, temperature, etc. More experimental work is needed to determine the exact nature of the agency causing these radiations.

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[1] Russell, W. J., *Proc. Roy. Soc.*, **61**, 424 (1897).
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We wish to report the following experiments which were designed as a consequence of McLean's observations reported here.

In our experiments we also used X-ray film, Kodak 'Crystallex', which is a standard material for X-ray diffraction work. Two series of experiments were carried out. In the first, the film was exposed for various times to hydrogen peroxide vapour by placing it above a dish containing the liquid. The film was then processed in the normal way. An example is shown in Fig. 1. (Compare with Fig. 1 of McLean.) In the second series of experiments a film which was exposed to hydrogen peroxide vapour, but not developed, was placed in contact with a fresh film. The two films were then developed and a transfer effect was observed as can be seen in Fig. 2*a* and 2*b*. (Compare with Fig. 3 of McLean.) We believe that the vapour particles attached to the original film were transferred to the second, resulting in the same effect as in the first series of experiments.

Fig. 1 Fig. 2*a* Fig. 2*b*

Fig. 1. Film exposed to hydrogen peroxide vapour for 1 hr.
Fig. 2*a*. As in Fig. 1
Fig. 2*b*. 'Transfer' from 2*a*. Time of transfer, 22 hr.

We made no attempt to reproduce McLean's 'transmission' effect using hydrogen peroxide; little information is given in the original work. It would be interesting, for example, to have such data as the porosity of the aluminium foil used, also the relative intensities obtained.

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Initial Vibrations of a Supported Beam

The theory of steady flexural vibrations of a supported beam is well known, but that concerning the initial vibrations produced by impact seems to have received little attention. We have therefore carried out experiments on the subject.

Flexural vibrations of metal strips were initiated by striking the strips normally to the greatest area with pendulum-type hammers. The vibrations were examined by using a variable-reluctance transducer coupled through an amplifier to an oscilloscope. The movable component of the transducer was fixed to the beam or was formed by the beam itself when steel ones were used. The conversion characteristics of the transducer were found to be nearly constant with frequency for the small amplitudes of vibrations obtained in the experiments. The metal beams were all 2·54 cm. × 0·318 cm. × 40 cm., and were supported on knife-edges placed as closely as possible to their ends. Hammers made of various materials were used and were shaped to give approximately point contacts on the beams. Soft hammers, such as those made from cork or rubber, were found to result in a single impact, as shown by George[1] and Cliffe[2] for soft hammers striking pianoforte strings.

Figs. 1 and 2 show the oscilloscope trace obtained when a mild-steel beam was struck by a soft hammer at its centre, where the transducer was also located on the reverse side of the beam. In Fig. 1 the small timing pulses are spaced at intervals of 20 msec.

while in Fig. 2 the pulse spacing is 2 msec. In these figures the first apparent half-cycle of the fundamental mode of vibration is clearly seen to be of smaller amplitude and of shorter duration than the subsequent half-cycles. The same features were found in many of the photographs obtained by altering the positions of the transducer and the impact and by using beams of different metals.

The impulsive force acting at the hammer is not known, and attempts will be made to elucidate the force–time relationship which exists during impact, but the anomalous first half-cycle can be explained by assuming a form for the impulsive force and then following the method of Carslaw and Jaeger[3]. The force P_t acting at the centre of the beam due to the hammer is assumed to be given by $P_t = P_0(\exp(-at) - \exp(-bt))$, where P_0, a and b are all large positive constants and P_t is the force at time t. Fig. 3 shows graphs of $\exp(-at)$ and $\exp(-bt)$ from which a graph giving $(\exp(-at) - \exp(-bt))$, and hence the form of the impulsive force, can be constructed. Such a form agrees with that suggested by Goland *et al.*[4], although the ways in which impacts were obtained in this earlier work are different from those in the present experiments.

The displacement y_t of the centre of the beam at time t is then given by :

$$y_t = A_1\cos(kt) + B_1\sin(kt) + A_3\cos(9kt) + B_3\sin(9kt) + A_5\cos(25kt) + B_5\sin(25kt) + \ldots + A_n\cos(n^2kt) + B_n\sin(n^2kt) + \ldots - C\exp(-at) + D\exp(-bt) \quad (n \text{ odd})$$

where k, A_n, B_n, C and D are positive constants. k depends on the elasticity and dimensions of the beam. If $y_t = 0$ at $t = 0$, A_n, C and D are related by:

$$A_1 + A_3 + \ldots + A_n + \ldots - C + D = 0$$

Other considerations suggest that the amplitudes A_n and B_n are inversely proportional to the square of n. Thus, a simplified synthesis of y_t using only the terms

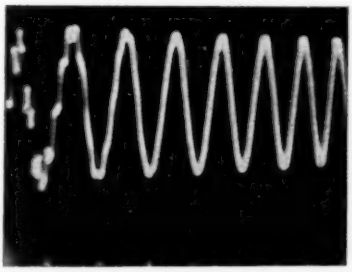

Fig. 1. Oscilloscope trace, timing pulse spacing 20 msec.

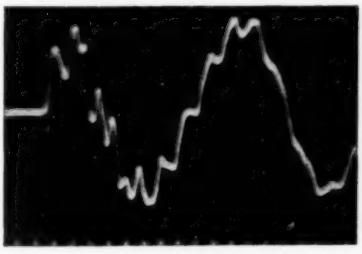

Fig. 2. Oscilloscope trace, timing pulse spacing 2 msec.

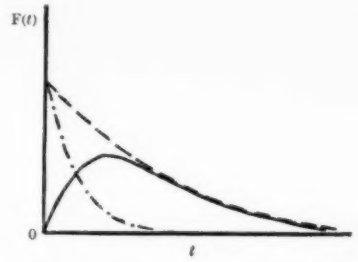

Fig. 3. Graphs of $\exp(-at)$, $\exp(-bt)$ and $\exp(-at) - \exp(-bt)$. — —, $\exp(-at)$; — · —, $\exp(-bt)$; ——, $\exp(-at) - \exp(-bt)$

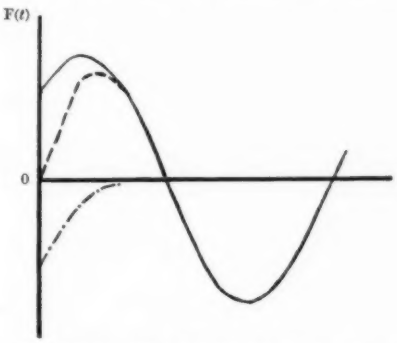

Fig. 4. Graphs of $A_1\cos(kt) + B_1\sin(kt)$, $D\exp(-bt) - C\exp(-at)$, and $A_1\cos(kt) + B_1\sin(kt) + D\exp(-bt) - C\exp(-at)$. ——, $A_1\cos kt + B_1\sin kt$; — · —$D\exp(-bt) - C\exp(-at)$; - - -, $A_1\cos kt + B_1\sin kt + D\exp(-bt) - C\exp(-at)$

having coefficients A_1, B_1, C and D may be expected to yield a fair representation of the actual y_t.

Such a synthesis is given in Fig. 4 in which the sine and cosine terms have been combined. This figure shows a marked similarity to Figs. 1 and 2 so that the assumption of the form of P_t is further justified. Additional justification could perhaps be obtained by analysing wave-forms such as those shown in Figs. 1 and 2, but the analysis is made difficult by the presence of the anomalous first half-cycle.

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Ultrasonic Absorption of Polymer Solutions

It is well known that the presence of a small amount of low-absorption solute in a highly absorbing solvent will reduce the absorption of the solution by much more than would be expected from simple proportionality. This is the case for mixtures of benzene (high absorption) with methyl methacrylate (low absorption). Wada and Shimbo[1] have shown that if polymethylmethacrylate is added to a benzene–methylmethacrylate mixture the resultant absorption is higher than in the absence of the polymer, although

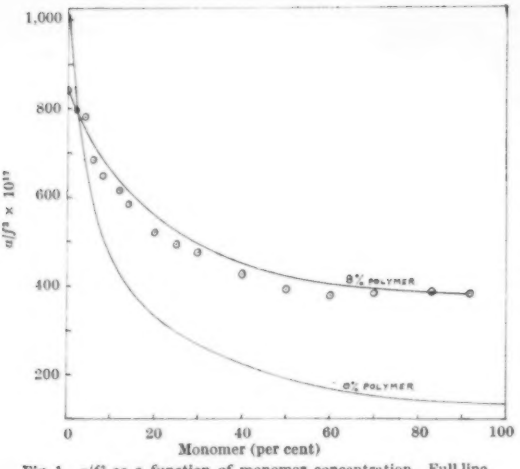

Fig. 1. α/f^2 as a function of monomer concentration. Full line, labelled 8 per cent polymer, predicted curve; dotted circles, experimental points

remaining less than that for the pure benzene. They suggested that this rise in absorption is caused by some of the monomer becoming 'enclosed' within the polymer chains so that it is no longer effective in reducing the solution's absorption. It is possible, however, to account for this rise in absorption without assuming 'enclosure'.

It is assumed that the absorption of a polymer – monomer – benzene mixture is made up of: (*a*) the absorption of a monomer – benzene mixture in which the polymer behaves in a similar manner to monomer in reducing the absorption; (*b*) an absorption due to the viscous motion of the solvent relative to the polymer chains[2,3]. This viscous absorption is assumed to be directly proportional to the mass of polymer present and the viscosity of the solvent. It is further assumed that these absorptions are additive. On these assumptions it is possible to account for the effects of the polymer found by Wada and Shimbo.

The ultrasonic absorptions of the mixtures have been measured at 26° C. and 5·19 mc./s. by a standard pulse technique. The measured absorption of 'Analar' benzene (expressed as α/f^2) is $1{,}010 \times 10^{-17}$, which is higher than the generally accepted value of 900×10^{-17}. However, for the present purposes, this discrepancy is unimportant since all the absorptions were measured under identical conditions with the same equipment.

The absorption due to the viscous motion of the solvent relative to the polymer chains was measured by comparing the absorption of a solution of polymethylmethacrylate in methylmethacrylate monomer with that of the pure monomer. Using monomer as solvent should probably eliminate any impurity effect which the polymer might have on the absorption. Expressed in terms of α/f^2 the viscous absorption of the polymer in monomer is 31×10^{-17} per gm. of polymer in 100-ml. solution. Thus, allowing for the higher viscosity of benzene compared with monomer, the expected value of α/f^2 for an 8 per cent (w/v) solution of polymer in benzene, in the absence of an impurity effect lowering the absorption, should be $1{,}310 \times 10^{-17}$ while the measured value is 850×10^{-17}. The difference of 460×10^{-17} is that which would be produced by 6 gm. of monomer in 100 ml. of benzene solution. For 6 per cent and 4 per cent polymer solutions the corresponding equivalent monomer concentrations are found to be 3·8 gm. and 2·6 gm. of monomer in 100 ml. of solution. It appears, therefore, that the efficiency of the polymer, in so far as reducing the absorption is concerned, is about two-thirds that of the monomer (the actual values are 0·75, 0·63 and 0·65 respectively). This seems to be reasonable since the reduction in absorption by an impurity is due to collisions between it and the benzene molecules. If the monomer molecule is regarded as a cube there are six faces available for a collision with a benzene molecule, but when the monomer is connected into a polymer chain the number of faces available for collision drops to four for each monomer unit.

From a knowledge of the variation in α/f^2 and viscosity with monomer concentration in benzene – monomer mixtures and the amount of monomer equivalent to the polymer so far as reduction of absorption is concerned, it is possible to predict the variation in α/f^2 with monomer concentration for polymer – monomer – benzene mixtures. The results of one such calculation for an 8 per cent (w/v) solution of polymer are given in Fig. 1. The predicted curve lies consistently above the experimental points, showing that although the theory gives moderately good results it still requires improvement.

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Molar Sound Velocity in Molten Hydrated Salts

From a systematic study of the temperature-variation of ultrasonic velocity and density in organic liquids, Rao[1] has found that the ratio of the temperature coefficients of the ultrasonic velocity and density is nearly equal to 3 for most organic liquids. From this result he has deduced the relation:

$$R = \frac{M}{\rho}(V)^{1/3}$$

where M is the molecular weight, ρ is the density, V is the ultrasonic velocity and R, known as molar sound velocity, is characteristic of the liquid and is independent of temperature. Although Rao established this relation in the case of organic liquids, later workers[2] have extended it to several inorganic liquids and have shown that R is independent of temperature. In the present communication, results are given of the variation with temperature of ultrasonic velocity in five molten hydrated salts.

The substances studied were lead acetate, sodium acetate, sodium–potassium tartrate, aluminium nitrate and calcium nitrate. For these substances the ultrasonic velocities in the molten state have been measured at various temperatures by using the fixed path interferometer method[3]. The variation of ultrasonic velocity with temperature for all salts is shown in Fig. 1. It will be seen that the variation of ultrasonic velocity with temperature is non-linear in all the salts except sodium acetate, which shows a linear decrease with increase of temperature. While

investigating the variation of ultrasonic velocity with temperature in aqueous solutions of certain inorganic sulphates, Marks[4] found that the velocity–temperature curves are similar to those of water but displaced towards lower temperatures with increase in concentration. In the light of this earlier observation, the curves for the melts of sodium and lead acetates and calcium nitrate appear to be similar to the latter half of velocity-temperature curves of highly concentrated aqueous solutions, the maxima of which have been displaced considerably towards lower temperatures. It is interesting to note that the velocities in molten lead acetate are less than that of water. This behaviour is similar to that of other aqueous solutions of lead salts, as reported by Bärthel[5] and Krishnamurty[6]. The most unusual variation of ultrasonic velocity with temperature is observed in the case of double salt sodium–potassium tartrate, which shows a maximum at 75° C. which is very near the temperature of maximum velocity of water. A similar maximum is observed less prominently in the case of melt of aluminium nitrate. The behaviour of both these salts is unusual and needs further investigation in detail.

The values of molar sound velocity R are calculated at different temperatures, and it is found that for each salt R is independent of temperature. The maximum change, which is about 2 per cent, is observed only in the case of sodium–potassium tartrate. It may be noted that this temperature-independence of molar sound velocity has not been reported previously. Considering the molten hydrated salt as a highly concentrated aqueous solution of the salt in its own water of hydration and assuming linear variation of R with concentration at least in the higher concentration-range, the values of R for each salt are obtained by extrapolating to 100 per cent concentration and these values are given in Table 1. Computed values of R for the first three salts using previously estimated[7] values of R for the individual elements showed good agreement with the experimental values, thus justifying the procedure adopted here for calculating R for the pure salts from those of molten hydrated salts. The values of R for the last three salts in Table 1 are utilized for calculating R values for the metals aluminium, calcium and lead and have been given in the last column. It may be noted that the values of R for these three metals are obtained for the first time.

Table 1

Salt	Cm. (per cent)	R for hydrated salt	R for dehydrated salt	Computed value for salt	Computed value of R for new element
Sodium acetate	25	315	634	680	—
Sodium–potassium tartrate	20	438	1,350	1,329	—
Aluminium nitrate	10	295	1,060	—	Al = 319
Calcium nitrate	25	391	934	—	Ca = 436
Lead acetate	25	412	1,018	—	Pb = 127

We are indebted to the Council of Scientific and Industrial Research for financial assistance of this research work.

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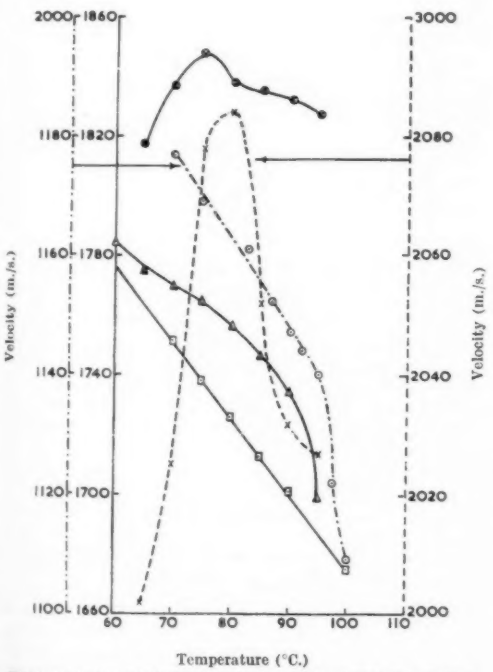

Fig. 1. Variation of ultrasonic velocity with temperature in molten hydrated salts. ⊗, Aluminium nitrate; ×, sodium potassium tartrate; △, calcium nitrate; ○, lead acetate; □, sodium acetate

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Neutron 'Flux'

Mr. Stretch[1] objects to the use of the term 'neutron flux' because the entity specified is a scalar, the movement of neutrons involved being random instead of a directed flow ; he contrasts this with the usage of the word 'flux' for a vector quantity. This usage, as in 'heat flux', is, however, already a degenerate form. Though now often used with a vectorial meaning, 'flux' was originally a scalar, albeit derived from a vector. The original meaning, which is still the customary one in electrical theory, is 'the surface integral of the normal component of a vector', and this is a scalar. It is this mathematical concept, surely, that should be the standard of orthodoxy.

The suggestion of replacing 'neutron flux' by 'neutron potential' is open to the objection that it already has a different meaning when considering the interaction of a neutron with another particle.

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MINERALOGY

Basic Dyestuffs in Clay Mineralogy

Basic dyestuffs are adsorbed on clay minerals by cation exchange reaction. The amount of dye adsorbed[1] and the resulting colour transformations[2] provide a possible basis for identifying clay minerals, but they are not very reliable. We have found that clay mineral – dye complexes exhibit characteristic thermograms and this method may possibly be applicable to the identification of clay mineral. Certain clay minerals which exhibit similar thermograms can be differentiated by the thermograms of their dye complexes.

Four types of clay minerals, namely, kaolinite, illite, nontronite and montmorillonite, were each treated with excess of aqueous solution of malachite green, methylene blue, methyl violet or piperidine. The unadsorbed organic material was removed by filtration and thorough washing. Thermograms were obtained by carrying out the differential thermal analysis of the complexes semi-automatically, using a Leeds and Northrup programme controller. The rate of heating was maintained at 10 deg. C./min.

Thermograms of kaolinite, illite, nontronite and montmorillonite with three basic dyestuffs, namely, malachite green, methylene blue and methyl violet, have been investigated. Differential thermal analyses of piperidine complexes of clay minerals have also been carried out for comparison. All the complexes exhibit low-temperature exothermic peaks at about 100° C. smaller than those of the untreated samples. The organic matter probably occupies the spaces normally held by the water molecules.

Kaolinite–dye complexes exhibit a small unmistakable exothermal dent between 350° and 435° C. due to the oxidation of a small amount of dye adsorbed by base-exchange reaction. The exothermic peak of kaolinite–malachite green complex is much more intense than the other kaolinite complexes, because malachite green is adsorbed to a greater extent than other dyestuffs.

Illite – dye complexes (Fig. 1*a*) show two intense exothermic peaks between 200° and 500° C. Nontronite and montmorillonite – dye complexes (Fig. 1*b*) show three exothermic peaks, two of pronounced intensities.

The second exothermic peak in illite-complexes at 460°–470° C. is less intense than that of nontronite-complexes occurring at about 600° C. Illites can be differentiated readily from nontronites by the second exothermic peak.

In montmorillonite – dye complexes, the higher-temperature exothermic peak at 650°–670° C. serves to differentiate montmorillonites from nontronites and illites, in which it occurs at about 600° C. and 460°–470° C. respectively.

From differential thermal analysis and thermogravimetric analysis it is concluded that low-temperature exothermic peaks are caused by oxidation of the dye molecules adsorbed on the edges of the clay minerals, while the high-temperature exothermic peaks are due to oxidation of dye molecules adsorbed in the interlayer.

Thus differential thermal analysis of clay mineral – dye complexes provides a more efficient method of identifying clay minerals than that of the clay mineral – piperidine complexes suggested by Allaway[3], Byrne[4] and Carthew[5]. Such complexes exhibit pronounced exothermal inflexions. In addition, the kaolinite – dye complex also shows a distinctive exothermal dent.

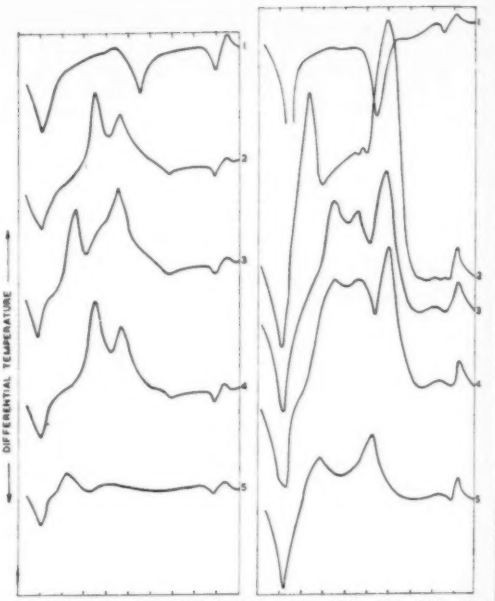

Fig. 1. (*a*) Thermograms of illite and its complexes: (1) illite; (2) malachite green complex; (3) methylene blue complex; (4) methyl violet complex; (5) piperidine complex. (*b*) Thermograms of nontronite and its complexes: (1) nontronite; (2) methylene blue complex; (3) malachite green complex; (4) methyl violet complex; (5) piperidine complex

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GEOCHEMISTRY

Hot Spring Areas with Acid–Sulphate–Chloride Waters

White[1], in a review of the thermal waters of volcanic origin, pointed out the problem of springs with a large flow of acid–sulphate–chloride water, such as Frying Pan Lake (or more correctly, Echo Crater) in the Waimangu area of the Rotorua-Taupo graben. This has a daily discharge of 800 kgm. of sulphuric

acid. White remarked that the surface oxidation of hydrogen sulphide to sulphuric acid in the type of porous ground favourable to this process could account for only an insignificant part of the total production of sulphuric acid. Typical hot underground chloride water, as at Wairakei, is nearly neutral in reaction, and it seems unlikely that water appreciably acid could be stored underground without attack on the country rock. This communication suggests an explanation for the high-output acid–sulphate–chloride type of spring.

The second dissociation constant of sulphuric acid falls rapidly with rise of temperature. From data given by Noyes[2] from conductance determinations on 0·01 molar sulphuric acid up to 218° C., it appears that the second dissociation constant for 0·01 molar acid rises from approximately 5×10^{-6} at 250° C. to 0·019 at 20° C. If the water is neutral at 250° C., it will become acid at ordinary temperatures owing to increased dissociation of bisulphate by the reaction $HSO_4^- = H^+ + SO_4^{2-}$. The fall in *p*H due to this will outweigh the effect of the loss of carbon dioxide in the steam escaping from water reaching the surface. This means that sulphate waters which are neutral in *p*H underground at elevated temperatures can become waters of low *p*H at the surface.

Recently, chemical data have become available for the Rotokawa thermal area, about five miles east of Wairakei. This is an area with waters of acid character (*p*H about 2·5) containing both chloride and sulphate. Analyses of a number of hot-spring waters from Rotokawa indicate that there are two water types having chloride contents of 1,600 and 750 p.p.m. and sulphate contents of 400 and 1,000 p.p.m., respectively. The ionic ratios, chloride/sulphate, are about 10 and about 2 respectively. If the sulphate were of surface origin a random distribution of ratios would be expected. It is thought that both waters are supplied from deep underground sources.

It can be calculated for the Rotokawa examples that the *p*H of deep neutral waters (*p*H 5·65) at 250° C., containing 270 p.p.m. and 700 p.p.m. of total sulphate, would, after losing 30 per cent of steam at the surface, and cooling to 20° C., change in *p*H to 2·95 and 2·6 respectively. This is on the assumption that the content of bicarbonate in the underground water can be neglected. These *p*H values can be compared with those for a number of Rotokawa spring waters of low ammonia content. Three of the low-sulphate type had an average *p*H of $2·9 \pm 0·4$, and seven of the high-sulphate type had an average *p*H of $2·3 \pm 0·15$.

It seems, therefore, that some areas of acid springs of mixed chloride–sulphate water do not differ essentially in their origin from areas with springs of so-called 'alkaline' water (better termed chloride water). As there has not been any drilling at Rotokawa, it is not possible to obtain the ratio of chloride to total sulphur (that is, to sulphur both as hydrogen sulphide and as the sulphate) in the underground water but from the sulphate content alone it is at most 10. At Wairakei the ratio is 60. From other areas it is known that there are large differences in the ratios of chloride to boric acid, carbon dioxide, etc.

Hence the occurrence of waters of high sulphate content and low *p*H involves two problems: first that of the presence of a large amount of sulphur as sulphate (though not necessarily a larger proportion of the total sulphur), and secondly that of the low ratio of chloride to total sulphur.

Two possible mechanisms for producing chloride water high in sulphate can be suggested. First, the waters could result from the reaction of hydrothermal solutions with old sulphur deposits formed by oxidation of hydrogen sulphide near the surface, and buried by later volcanic activity. Disproportionation of sulphur in neutral solutions to sulphate and sulphide is predicted by thermodynamic information[3] and occurs readily in the laboratory.

The second mechanism is that hydrogen sulphide is oxidized to sulphate by ferric iron in the rocks. If the partial pressure of hydrogen in water in contact with iron-containing rocks is defined by the coexistence of hæmatite and magnetite, for equilibrium at temperatures over 250° C. in waters of neutral *p*H the sulphate/sulphide ratio is high[3]. This assumes that conditions prevailing correspond to a point on the phase diagram[4] where iron oxides rather than sulphides are the stable phases. For this to be the case at the likely hydrogen sulphide pressures of around 10^{-2} atmosphere, high temperatures probably more than 300° C. are necessary. Although iron sulphides become stable at lower temperatures as the water approaches the surface and loses steam, reversion of sulphate to sulphide is a slow process, and will not cause the loss of much sulphur from solution.

The first process would explain the low ratio chloride/total sulphur but is a less-likely occurrence. The second explanation makes the problem, apart from the possible requirement of high temperature, part of the general problem of the occurrence of deep chloride waters with great differences in the ratios of chloride to total sulphur, to carbon dioxide, to boric acid and to fluoride.

The analyses of the waters were made by Mr. W. A. J. Mahon, of the Dominion Laboratory.

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BIOPHYSICS

An Invariant Casein Substrate for Rennet Standardization, derivable from Diverse Sources of Cows' Milk

When rennin or commercial rennets (impure rennins) cause milk to coagulate, it is well known that the primary enzyme action consists in decomposing a part only of the casein, known as κ-casein[1]. Later, either through the activity of the products of decomposition or through loss of protective action, the casein aggregates and finally a gel (curd) is formed.

We have recently shown[2] that the initial decomposition as indicated by the fall in viscosity (Ostwald viscometer) follows a first-order equation under normal conditions. (At the concentrations used, all solutions are Newtonian.) The logarithmic reaction constant (k) is proportional to enzyme concentration when pure crystalline rennin is used, but varies as a power ($N < 1$) of the concentration for commercial

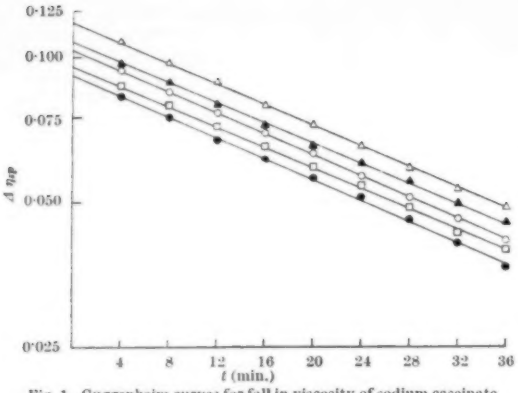

Fig. 1. Guggenheim curves for fall in viscosity of sodium caseinate from various milk sources: rennet *A*. △, Jersey; ▲, Friesian; □, Ayrshire; ○, bulk milk *A*; ●, bulk milk *B*

rennets. It seems likely that N reflects the purity of the rennet whereas k should give a measure of its activity. This latter may be high even for impure rennets, due to the presence of enzymes other than rennin. The problem is to find a reproducible substrate against which to compare different rennets, since milk, being a biological fluid, does not have a fixed composition; for example, milks may well differ in their proportions of κ-casein. The question is whether the composition of the κ-casein also varies.

We have found it possible to prepare sodium caseinates from milks not only from different herds but also from different breeds of cow, which give, within the limits of experimental error, the same value of k for any one rennet but, in general, different values for different rennets. These caseinates have the additional advantage that they show no appreciable aggregation.

The caseinate is prepared by finely precipitating it from fat-free milk at the isoelectric point (*p*H 4·7) with N hydrochloric acid, and then removing as much as possible of the soluble milk components by frequent washing and filtering. The acid casein is dissolved by the slow addition of 0·5 N sodium hydroxide, and vigorous stirring, taking care that the mixture does not become alkaline. Stirring is continued for at least 2 hr., with excessive acid casein present, before the concentrated sodium caseinate solution, which should now have a *p*H of 6·2–6·4, is filtered and freeze-dried. The experimental samples, made up from the freeze-dried sodium caseinate, are adjusted for *p*H and then heated to 80° C. for 5 min. to destroy the natural proteases. They are then kept for another 24 hr. in the cold to regain their normal viscosity, reduced during the heating. Before use it is also desirable to filter the substrate through suitable sinter-glass without suction. Unless these precautions are taken in preparing the caseinates, the values of k for different samples, using the same rennet, may differ considerably.

To determine k, a smooth curve is drawn by inspection through the graph of η_{sp}/time (where η_{sp} is the specific viscosity). There are normally some 20 points on each experimental curve. The time zero is the moment when rennet is added. Differences of η_{sp} ($\Delta\ \eta_{sp}$) corresponding to a standard time-interval ($\Delta\ t$) are determined, following Guggenheim's method[3]. The slopes of the Guggenheim curves, plotting log ($\Delta\ \eta_{sp}$) against t, give the values of k, only with a change of sign.

The positions of the curves would depend on the proportional falls in viscosity produced by the enzymes, that is, on the concentrations of κ-casein, except that, since viscosity does not vary linearly with caseinate concentration, an empirical adjustment is made to give the different samples approximately the same initial viscosities. Since the contribution of the products of decomposition of κ-casein to the total viscosity is not known, the proportion of κ-casein cannot be directly calculated, though it appears to be at least 20 per cent, and often more, that is, considerably higher than was previously supposed[1]. In Fig. 1 are shown a number of Guggenheim curves for sodium caseinates prepared from milks from different breeds and from mixed herds. It is clear that for some 50 min. at least, the reaction is very close to first-order. If the experiments are prolonged, the curves tend to deviate from linearity.

Table 1

Source of caseinate (cows' milk)	$k \times 10^4$ sec.$^{-1}$	
	Rennet *A*	Rennet *B*
Jersey	4·06	3·83
Ayrshire	4·01	3·87
Friesian	4·09	3·89
Bulk *A*	4·19	3·94
Bulk *B*	4·14	3·80
Bulk *C*	4·05	3·90
Bulk *D*	4·09	3·87
Mean	4·09	3·87
Standard deviation	0·0597	0·0458

In Table 1 are given values for k, together with their means and standard deviations, for caseinates from seven milks for two well-known brands of commercial rennet. Rennet *A* has a higher value of N and is known to be purer in rennin than is rennet *B*, but both are considered satisfactory for cheese manufacture.

We conclude that sodium caseinate, prepared as described, may be reproduced from milks from widely different sources and hence probably over long periods of time. The value of k for a particular rennet should thus provide a stable index of its activity.

A full account of this work, including details of experimental conditions, will be submitted, in due course, for publication (*J. Dairy Res.*).

We are indebted to our colleagues at the Institute for many helpful discussions.

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Changes of Permeability of Yeast with Sodium Deoxycholate

Understanding of the phenomenon of cell permeability depends on detailed knowledge of the structure of the cell membrane. But the morphological and chemical organization of the cell membrane is still largely unknown. Study of the mechanism of action of detergents on the cell may represent one of

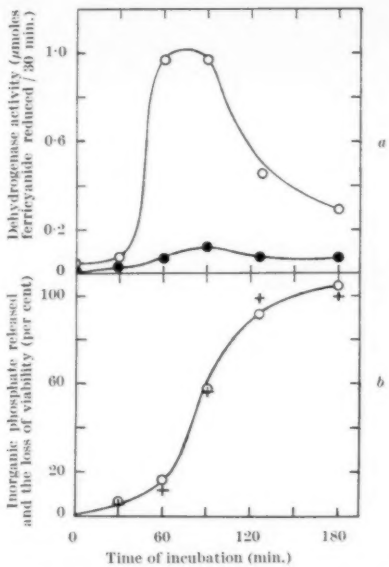

Fig. 1. Effect of deoxycholate on the dehydrogenase activity, inorganic phosphate released and viability in a suspension of yeast. Baker's yeast (11·7 mgm. dry weight/ml.) was incubated in 0·04 *M* *tris*-hydrochloric acid *p*H 7·5 with 0·006 *M* sodium deoxycholate at 27° C.; at suitable intervals, the medium was analysed for inorganic phosphate and the cells for succinic dehydrogenase activity. A portion of the cells was diluted and plated on Petri dishes with a synthetic medium; from the number of colonies the fraction of dead cells was calculated. *a*: ●, Dehydrogenase activity without added substrate; ○, succinic dehydrogenase activity; *b*: ○, inorganic phosphate released from the cells (per cent) (released by extraction with 10 per cent trichloracetic acid = 100 per cent); +, dead cells (percentage of total)

the suitable ways for the solving of this problem. From this point of view, the mechanism of action of deoxycholate on the yeast cell was investigated.

The intact yeast cells are not permeable to ions of dicarboxylic acids or to ferricyanide, hence no succinic dehydrogenase activity with ferricyanide as an electron acceptor can be demonstrated in the suspension of intact yeast cells. If deoxycholate is put into such a suspension, succinic dehydrogenase activity can be measured after a certain lag period; this means that in the presence of deoxycholate the cells become permeable. This effect of deoxycholate on the permeability is strongly dependent on temperature, increasing at higher temperature. The effect is enhanced by the lowering of *p*H (down to the point where the insoluble acid is precipitated) and by increasing ionic strength.

The dependence of the effect on the concentration of deoxycholate has a sigmoidal character. Under the experimental conditions used (see legend to Fig. 1), concentrations lower than 0·002 *M* were ineffective; at 0·005 *M* the effect was already maximal. At lower *p*H or higher ionic strength, the deoxycholate was effective even at lower concentrations.

Fig. 1 shows a time-course of the change in permeability of the cells incubated in the presence of deoxycholate, as measured by succinic dehydrogenase activity. The inorganic phosphate released from the cells and the viability of the cells are also plotted. Activity of succinic dehydrogenase reaches a maximum and then diminishes because of irreversible inactivation. Most striking is the fact that, while the loss of inorganic phosphate runs parallel to the loss of viability, which indicates that inorganic phosphate is released from the dead cells only, in a certain definite phase only about 20–30 per cent of cells are dead, while the succinic dehydrogenase activity measured is already a maximum. This suggests that part of the cells with the loose permeability barrier may be viable. This is supported, too, by the following observation: when the cells were pre-incubated with deoxycholate for such a time as to show almost a maximal or just a maximal succinic dehydrogenase activity and then washed with water, they did not show any, or only a very weak, succinic dehydrogenase activity, if deoxycholate were omitted from the test mixture. Thus, the action of deoxycholate may be, at least partly, reversible.

Recently, evidence has been provided by X-ray diffraction investigations[1], and direct observation by electron microscopy[2], that, under appropriate conditions, deoxycholate aggregates in solution to form a complex of macromolecular dimensions, the molecules assuming an elongated helical configuration. The dependence of the formation of these complexes on *p*H, on ionic strength and on the concentration of deoxycholate[3] is strikingly similar to the dependence of the changes of yeast permeability with deoxycholate on the above factors. This suggests strongly that the marked biological effect of deoxycholate, at least so far as its effect on yeast permeability is concerned, may be due to its ability to form helical fibre complexes. In these fibres, the hydrophobic steroid part of the molecule appears to be located inwardly, negatively charged carboxyl groups being on the surface. This seems to exclude the possibility that the molecules of deoxycholate would be oriented with their hydrophobic parts to the surface of the cell membrane and that the primary action of deoxycholate would be related to lowering of the surface tension. Rather there might exist another type of interaction, for example, similar to that which has been described recently by Lawrence[4], to explain the cleaning action of ionic detergents; that is, the interaction of the polar part of the deoxycholate complex with polar groups on the cell surface which would result in the formation of the ternary mixture, namely, polar part of deoxycholate/water/polar groups of the cell surface, followed by loosening of some components of the membrane, which are responsible for its permeability barrier.

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CHEMISTRY

Discreteness-of-Charge Effect and the Stability Theory of Colloids

ACCORDING to the stability theory of hydrophobic colloids of Derjaguin and Landau[1] and Verwey and Overbeek[2] (D.L.V.O. theory) the electrolyte concentration required to produce coagulation increases with the surface potential of the colloidal particles. However, for silver halide sols, the electrolyte concentration increases with surface potential for uni-

valent flocculating ions, either decreases or remains approximately unchanged for divalent ions and decreases for trivalent ions[3,4]. This can be explained by incorporating into the D.L.V.O. theory the so-called discreteness-of-charge effect[5–8].

Using the electric double-layer model of Grahame[9] and Devanathan[10] for a plate-like particle, an 'inner region' of oriented water dipoles, of thickness $d(\sim 5$ Å.) and dielectric constant ε_1 (~ 10–20), is assumed to lie between the particle medium and the dispersion medium, of dielectric constants ε_p and ε ($\sim$80), respectively. The volume density in the dispersion medium of the potential-determining ions, which constitute the charge on the particle wall and are negative, is small compared with the corresponding density n of the coagulating electrolyte of a z–z valency type. A layer of cations, valency z, is absorbed on the inner Helmholtz plane (I.H.P.), inside the inner region at a distance $\beta < d$ from the plate wall. The outer Helmholtz plane (O.H.P.), at distance d from the wall, is the boundary between the inner region and the diffuse layer. The two zones of the inner region of thicknesses β and $\gamma = d - \beta$ act as condensers of capacities $K\varepsilon/\beta$ and $K\varepsilon/\gamma$ per unit area, where $K = \varepsilon_1/4\pi d$. The mean electrostatic potentials at the wall, the I.H.P. and the O.H.P., are denoted by ψ_0, ψ_β and ψ_d respectively. Also σ_0 and σ_β are the mean surface densities at the wall and the I.H.P. Then we have the relations: $\psi_0 - \psi_\beta = \beta\sigma_0/K\varepsilon$, $\psi_\beta - \psi_d = \gamma(\sigma_0 + \sigma_\beta)/K\varepsilon$, $\sigma_0 + \sigma_\beta + \sigma_d = 0$, where σ_d is the total charge in a column of the diffuse layer of unit cross-section. Applying the Gouy–Chapman theory to the diffuse layer:

$$\sigma_d = -\left(\frac{2n\varepsilon kT}{\pi}\right)^{1/2} \sinh\left(\frac{1}{2}\frac{ze\psi_d}{kT}\right)$$

where k, T and e have their usual meanings.

In the Stern theory of the double layer the energy of adsorption of the cation consists of a chemical term and an electrostatic term $ze\psi_\beta$ and the discrete nature of the ionic charge is ignored. In allowing for this effect, the Soviet authors[5,6,8] neglect the thermal motion of the adsorbed cations and replace the continuous charge distribution by a hexagonal array of discrete ionic charges. We adopt a simpler 'cut-off' approximation, suggested by Grahame[7], which is equivalent in principle. Each adsorbed cation is regarded as the centre of a circular area in the I.H.P. from which the uniform charge density σ_β is removed, of radius r_0 such that $\pi r_0^2\sigma_\beta = ze$. By considering the multiple electrostatic image reflexions in the plane of the plate wall and in the O.H.P., where the dielectric constant is discontinuous, the resulting change in the potential at the centre of the adsorbed cation can be expressed very nearly as:

$$\varphi_\beta = -\frac{\beta\gamma}{K\varepsilon d^2}\, g\sigma_\beta$$

where g is a factor depending on ε, ε_1, ε_p and n. If $\varepsilon_1 \ll \varepsilon, \varepsilon_p$ then $g \approx 1$, but in general $g > 1$. The potential φ_β is a two-dimensional analogue of the self-atmosphere potential in the Debye–Hückel theory of electrolytes. The contribution $ze\varphi_\beta$ to the energy of adsorption is not small and is responsible for the apparent variation of the specific adsorption energy with surface charge at the mercury-aqueous electrolyte interface[9].

If each adsorbed ion, retaining a portion of its hydration shell, occupies s adsorption sites, then employing the volume fraction statistics of Flory[11] and Huggins[12], the Stern adsorption isotherm is modified to read:

$$kT\ln[\sigma_\beta/(1 - s\sigma_\beta/N_s ze)^s] = -ze(\psi_\beta + \varphi_\beta) + kT\ln n + C$$

where N_s is the number of adsorption sites per unit area and C is a constant which includes the chemical term in the adsorption energy. From these relationships maxima in both $|\psi_\beta|$ and $|\psi_d|$ are found at $\sigma_\beta = \sigma_\beta^{(m)}$, where:

$$\sigma_\beta^{(m)}[1 - s\sigma_\beta^{(m)}/N_s ze]\,[1 + s(s-1)\sigma_\beta^{(m)}/N_s ze] = \frac{kT}{ze\varepsilon g}K\frac{d^2}{\beta\gamma}$$

The electrostatic work of adsorption of a cation is $-ze(\psi_\beta + \varphi_\beta)$, which increases steadily with plate potential. When the ratio $r = \sigma_\beta/\sigma_\beta^{(m)} < 1$, both $|\psi_\beta|$ and $|\psi_d|$ increase with $|\psi_0|$, but at larger coverages ($r > 1$) the variation of φ_β with ψ_0 predominates, causing $|\psi_\beta|$ and, therefore, $|\psi_d|$, to diminish. A maximum in $|\psi_d|$ was found experimentally at the mercury-electrolyte interface[13].

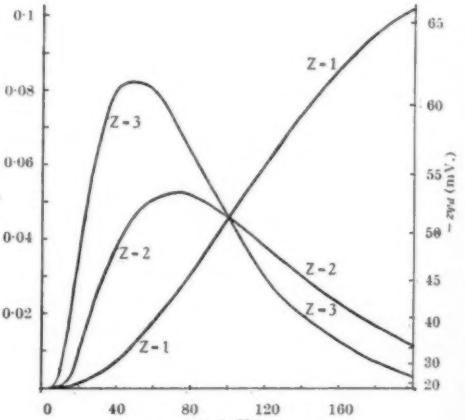

Fig. 1

Consider now two parallel plates of infinite thickness. If the potential distributions in the interpenetrating diffuse layers are linearly superposed, the coagulation condition becomes:

$$n = 107\,\frac{\varepsilon^3(kT)^5}{(ze)^6A^2}\,t^4,\quad t = \tanh\left(\frac{1}{4}\frac{ze\psi_d}{kT}\right)$$

where A is the constant in the van der Waals interaction energy. In the D.L.V.O. theory ψ_d is replaced by ψ_0 and, for large enough ψ_0, n is proportional to z^{-6}, a simple version of the Schulze–Hardy rule. In the typical plot of t^4 vs. ψ_0, by proper choice of C for $z = 1$, 2 and 3 this inverse sixth power rule is obeyed at $ze\psi_d/kT = 2$ and $r = 2$ for $z = 2$, where the three curves have a common intersection point ($\psi_d = 51{\cdot}4$ mV. if $z = 1$ and $T = 25°$ C.). Also $g = 1{\cdot}5$, $A = 2 \times 10^{-12}$ erg. cm.^{2}, $K = 30$ μF./cm.^{2}, $N_s = 5 \times 10^{14}$/cm.^{2}, $\gamma/d = 1/4$, $\varepsilon = 78{\cdot}5$ and $s = 1$, 3 and 5 respectively for $z = 1$, 2 and 3; the corresponding values of $\sigma_\beta^{(m)}$ are 2·84, 1·49 and 1·01 μcoul./cm.^{2}. The general trend of the dependence of coagulating concentration on plate potential is reproduced theoretically when $r < 1$ for $z = 1$, $r \gtrsim 1$ for $z = 2$ and $r > 1$ for $z = 3$.

Furthermore, deviations from the inverse sixth-power rule can occur, in agreement with experiment. The maximum in the $\psi_d - \psi_0$ curve, which is essential to the theory, is not obtained if the self-atmosphere

potential φ_β is omitted. Various refinements, such as variation of K and γ/d with z and ψ_0 and a correction to the van der Waals energy due to the difference between the medium of the inner region and the particle medium, should not affect the general argument.

Part of the work by one of us (S. L.) was carried out at the National Research Council, Ottawa, Canada.

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Permeation of Gaseous Oxygen through Vitreous Silica

THE permeation of gaseous oxygen through vitreous silica has been measured over the temperature-range 950°–1,080° C. in a permeation cell constructed with two portions separated by a thin-walled spherical bulb with a short neck. The bulb was 25 mm. outside diameter, and served as the membrane of vitreous silica ; its area was 18·9 cm.² and thickness 0·27 mm. The thickness was determined from electrical capacitance measurements. The silica was used as blown ; and no cleaning was done with hydrofluoric acid. The oxygen pressure, 300–800 mm. mercury, was applied to the convex side of the bulb. The low-pressure side led directly to a mass spectrometer with no leak intervening. The rate of permeation of gas was measured[1], in this case by the peak height of mass 32. It was assured that true permeation, and not merely degassing, was being measured by : (1) observing the slow rise of the oxygen peak after application of oxygen to the high-pressure side, (2) attainment of steady-state flow and then, (3) slow decay of the peak after the gas was removed from the high-pressure side.

The experimental values of rate of permeation P on an Arrhenius-type plot of log P against $1/T$ yielded a good straight line, giving the results shown in Table 1. The units of P are those used by Barrer[2] ; cm.³ gas (S.T.P.) per sec. for 1 mm. thickness per cm.² area per cm. mercury difference of gas pressure.

Table 1

Temperature (° C.)	Rate of permeation P
900	$9{\cdot}00 \times 10^{-13}$
950	$1{\cdot}25 \times 10^{-12}$
1,000	$1{\cdot}85 \times 10^{-12}$
1,050	$2{\cdot}50 \times 10^{-12}$
1,100	$3{\cdot}50 \times 10^{-12}$

From the slope of this plot an activation energy of permeation of 22,000 cal. per mole was derived.

The lag method of Daynes[3] and Barrer[2] is ideally adapted for the mass spectrometer to measure the rate of attainment of steady-state flow. By this method the diffusion constant D, in cm.²/sec., was found to be $1{\cdot}2 \times 10^{-8}$ at 1,078° C. and $4{\cdot}2 \times 10^{-9}$ at 950° C., yielding diffusion activation energy of 27,000 cal. for oxygen in vitreous silica. The lag time at 950° C. for the steady state was slightly more than 8 hr. in this particular cell with a wall 0·27 mm. thick.

The solubility was derived from the relationship $S = 7{\cdot}6\,P/D$, and found to be $1{\cdot}9 \times 10^{-3}$ at 1,078° C. and $2{\cdot}3 \times 10^{-3}$ at 950° C. The units are cm.³ gas (S.T.P.) per cm.³ solid for 76 cm. gas pressure applied.

The fact that molecular oxygen is the diffusing species is indicated by the observation that the rate of permeation was proportional to the first power of the pressure.

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Aniline Oxidation initiated by Ultrasonic Waves

IN order to explain the chemical processes occurring in the ultrasonic field, Lindström and Lamm[1] assumed that the principal reaction occurring during the ultrasonic treatment of water is its decomposition into radicals:

$$H_2O = H + OH$$

By means of this reaction it is possible to explain a number of chemical processes[2–6], the oxidation of aniline included.

Aniline solutions (0·5, 1, 2 and 3 per cent, Merck) were used. All the solutions were prepared in redistilled water. The ultrasonic treatment was carried out in identical chemical vessels, containing 50 ml. of the desired aniline solution. A magnetostrictive transducer of 5·1 cm.² radiation area was cooled by air in such a manner that the initial solution temperature (17°–18° C.) rose only by 4°–7° C., in accordance with the duration of the ultrasonic treatment. All the solutions were treated with the waves of 21-kc./sec. frequency and electric power of about 4·5 W./ml. of the solution.

All the aniline solutions of the appropriate concentration were treated with ultrasonic waves during 2 min. In the course of the treatment minute oxygen bubbles were let through every solution (about 35–40 bubbles per min.). After the treatment the solutions were poured into small calibrated cylinders with ground-in stoppers.

In the course of the observations it was found that the aqueous aniline solutions form different oxidation products after treatment as shown in Table 1.

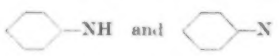
Table 1

Aniline solution concentration (per cent)	Ultrasonic treatment duration	Colour of dye
0·5	2 min.	violet
1·0	,,	violet-blue
2·0	,,	orange
3·0	,,	black shot with green

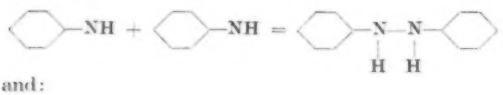
The violet dye obtained does not change under the influence of hydrochloric acid. Potassium hydroxide solution changes the colour of the dye to brown, but after acidification with hydrochloric acid it regains its violet colour. The violet-blue dye showed no changes under the influence of 6 per cent hydrogen peroxide solution. It separates on a filter paper into two layers: an external one of violet colouring and an internal one of black colouring shot with green. Neither of the dyes, violet and violet-blue, has been identified, but it is possible that they are derivatives of *p*-benzoquinone of an undetermined structure.

The orange dye has been identified as azobenzene, easily soluble in alcohol and especially in ether.

Concerning the azobenzene-forming mechanism in the ultrasonic field, it is necessary to assume first that aniline in aqueous solution decomposes into radicals:



which, being in an unsaturated state, react with each other according to the equations:



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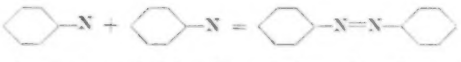
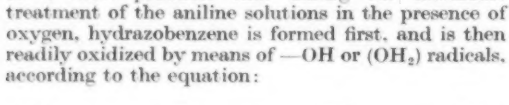


It is most probable that during the ultrasonic treatment of the aniline solutions in the presence of oxygen, hydrazobenzene is formed first, and is then readily oxidized by means of —OH or (OH_2) radicals, according to the equation:



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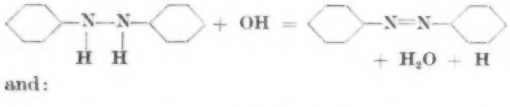
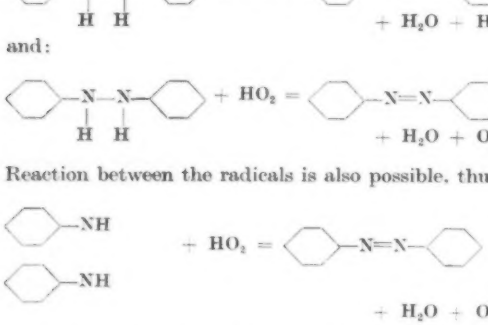


Reaction between the radicals is also possible, thus:



The black dye shot with green has proved to be a mixture of green dyes, the products containing less-oxidized aniline, and aniline black, not turning green when treated with hydrochloric acid.

The same quantities of the 3 per cent aniline solution were treated with ultrasonic waves during 1, 2, 3 and 4 min. respectively, at the temperature 17°–18° C. During the treatment, oxygen was let through the solution at the rate of about 35–40 bubbles per min.

In the course of the observations it was found that aqueous aniline solutions, during a short treatment (1–2 min.), formed mainly green products, probably emeraldine, nigraniline, and pernigraniline. After a longer treatment (3–4 min.) aniline black (not turning green) was mainly obtained as a poly-molecular product containing numerous quinonoid bonds.

In the course of oxidation of aqueous aniline solutions it was found that an increase in the molecular pressure of oxygen did not lead to a rise in the oxidation effect.

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Electron Affinity of the Acceptor Molecule in Charge-Transfer Complexes

Whereas the influence of the structure of the donor molecule in charge-transfer complexes on the stability and spectroscopic behaviour of these complexes has been investigated extensively, the role of the electron acceptor has been much less studied. One reason for this emphasis is the lack of measurements of a parameter related to the electron affinity of the acceptor molecule, in contrast to the fairly readily available ionization potentials of the donors.

It has been shown for alternant aromatic hydrocarbons that the one-electron polarographic half-wave potentials measured under conditions such that the mono-negative ions are virtually unprotonated are directly related to the energy of the lowest unoccupied molecular orbitals and hence the electron affinities of these molecules[1]. In general this may be expected to hold for any π-system, and the half-wave potential will at least be a measure of the readiness of the system to accept an electron[2].

A series of measurements has been carried out on well-known acceptors using acetonitrile and dimethylformamide as solvents; the polarographic characteristics of hydrocarbons and quinones are well established under these conditions[2]. The results given in Table 1 show the potential of the first one-electron addition; in each case it was confirmed by a.c. polarography and conventional means that the electron addition is sufficiently rapid not to require a measurable over-voltage and that no proton addition occurs. These compounds show a second one-electron step, some 0·8 V. more negative than the first; for simplicity this has not been included in Table 1. The figures in Table 1 are, in general, clearly in the expected order for complexing ability[3,4], the stronger complexing agents having the more positive potentials. The results for the two solvents are practically identical. The difference in complexing ability between chloranil and quinone is well brought out in contrast with a comparison of their conventional redox potentials (689 and 700 mV.). This method would thus appear to be at least a useful means of comparing acceptor-strengths. An indication that the series may provide more than a qualitative comparison of acceptor strengths is provided in Fig. 1 in which the change in free energy for complex formation with pyrene, determined spectroscopically[4], is plotted against the polarographic half-wave

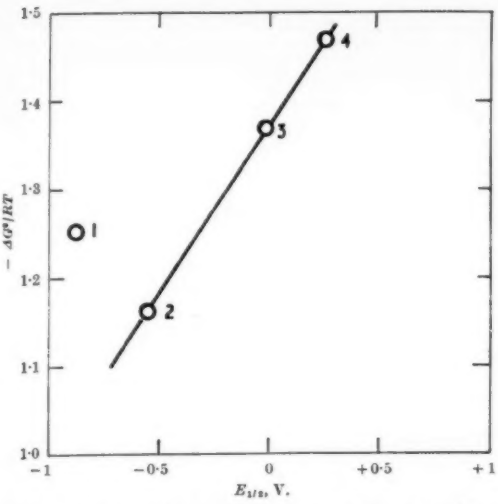

Fig. 1. Change in free energy of a series of complexes with pyrene (ref. 4) as a function of the half-wave potential of the acceptor. 1, Maleic anhydride; 2, *p*-benzoquinone; 3, chloranil; 4, tetracyanoethylene

potential of the acceptor. Other factors being equal, one would expect that the change in free energy would be directly related to the energy required to place an electron in the acceptor molecule. From Fig. 1 it would appear that the maleic anhydride complex has a greater stability than would be expected from the half-wave potential of this acceptor; pyromellitic dianhydride is also exceptional in this respect as it is claimed from spectroscopic measurements[5] that its complexing ability is close to that of tetracyanoethylene. An investigation into the reduction processes of anhydrides is in hand to see if further light can be thrown on this apparent anomaly.

The potentials of the second one-electron steps of the first three compounds in Table 1 lie at − 0·31, − 0·75 and − 0·80 V., in the region of the first steps for moderately strong acceptors. This suggests that it may be possible for a second electron to be shared with these molecules. This would apply more readily to compounds with even more positive half-wave potentials.

Table 1. HALF-WAVE POTENTIALS AT 25° OF THE FIRST ONE-ELECTRON ADDITION FOR SOME ORGANIC ACCEPTOR-MOLECULES. SUPPORTING ELECTROLYTE 0·1 *N* NEt_4ClO_4

Substance	Half-wave potential, *V*, versus S.C.E.*	
	Acetonitrile	Dimethyl-formamide
2 : 3 Dicyano 5 : 6 dichlorobenzo-quinone	+0·51	—
Tetracyanoethylene	+0·25	+0·24
Chloranil	+0·02	+0·02
Picric acid	−0·26†	—
p-Benzoquinone	−0·52	−0·54
Pyromellitic dianhydride	−0·60	−0·60
p-Xyloquinone	−0·67	−0·68
9 : 10 Phenanthrenequinone	−0·66	−0·70
2-Methyl naphtho 1 : 4 quinone	−0·78§	—
Duroquinone	−0·84§	—
Maleic anhydride	—	−0·88‡
Anthraquinone	−0·94	−0·98
Phthalic anhydride	—	−1·36†

*Including liquid junction potential, acetonitrile–water.
†Some irreversibility, true $E_{\frac{1}{2}}$ may be ± 0·05 V.
‡Two-electron change.
§From Wawzonek, S., Blaha, E. W., Berkey, R., and Runner, M. E., *J. Electrochem. Soc.*, **103**, 456 (1956); potentials converted to S.C.E. (saturated calomel electrode).

Further work is planned on the quantitative aspects of this approach to the problem of donor–acceptor complexes. If a quantitative correlation were established, it would provide a basis for a greater understanding of the factors contributing to the stability of the complexes. It would also appear feasible to investigate donor–acceptor equilibria by polarographic means in a manner analogous to that for metal-ion complexes[6] in which factors such as contact charge-transfer would not interfere.

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Determination of Indium in High-purity Zinc

THE activation analysis of 5 parts in 10^{12} of indium in zinc has been carried out, using a method of separation investigated by Kosta and Hoste[1]. 1-gm. samples of zinc were irradiated in a neutron flux of 8×10^{11} n./cm.^{2} sec. in the *BR.I* reactor. After irradiation, the samples were dissolved in 10 ml. of 6 *N* hydrobromic acid with the addition of 3–4 drops of nitric acid (sp. grav. 1·40) in order to accelerate the dissolution. By addition of 5 ml. of 6 *N* hydrobromic acid to the solution, its concentration was increased to about 4 *N*. After two extractions with 5 ml. of isopropylether, the organic layer contained all the trivalent indium. Small amounts of zinc-65, which were present in the organic layer, were washed out with 10 ml. of 6 *N* hydrobromic acid.

The purified indium fraction contained the radio-isotope indium-116*m* ($t1/2$ = 54 min.) and traces of zinc-65 ($t1/2$ = 240 days) and of zinc-69*m* ($t1/2$ = 14 hr.). Since discrimination of the gamma transitions of zinc-65 (E_γ = 1·114 MeV.) and of indium-116*m* (E_γ = 1·09 MeV.) is impossible by gamma-spectrometry, the activity of the former was determined by measurement of its 1·27-MeV. γ-ray. A single-channel gamma-spectrometer with a 3 in. × 3 in. sodium iodide (thallium) well-detector system was used for these measurements. The 438-keV. γ-ray from the isomeric transition of zinc-69 is used for measuring the purity of the indium fraction.

In order to avoid shadow effects of epithermal neutrons, the indium standards must be of very small size. Therefore one drop of an indium solution (0·2 mgm. indium/ml.) is weighed in a quartz tube and used as the standard. After irradiation and hundred-fold dilution, its level of activity is comparable to that of the indium in the samples. Results indicate the presence of 4·9–5·0 parts in 10^{12} of

indium in three zinc samples of different purity in respect to other impurities.

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Reactivity of Calcium Sulphate Hemihydrate

Calcium sulphate hemihydrate reacts with water to yield calcium sulphate dihydrate. The rate of this reaction differs considerably from sample to sample, the variations in reactivity being due, at least in part, to conditions prevailing during the dehydration of the parent dihydrate. We have investigated the reactivity of calcium sulphate hemihydrate prepared by calcining various samples of finely powdered reagent grade calcium sulphate dihydrate (44 per cent −100 mesh and +200 mesh, 54 per cent −200 mesh) spread as a thin layer (120 gm. per 500 cm.²) on a tray in an air oven. After calcination the material was exposed to the atmosphere at room temperature to allow absorption of moisture until the composition of hemihydrate was attained[1]. The reactivity of the product was assessed from the rate of increase in temperature of mixtures of 100 gm. of hemihydrate with 60 gm. of water, held in a Dewar flask[2]. The mixtures were made at 20° C. Two parameters of the reaction were studied, namely, the induction period defined as the time taken for the rate of increase in temperature to exceed 0·1 deg. C./min., and k the slope of the approximately straight part of the plot against time of $\log_{10}\Delta T/\Delta T_f$, where ΔT deg. C. is the increase in temperature and ΔT_f is the increase in temperature corresponding to complete hydration. The constant k is a measure of the self-acceleration of the reaction[1-3]. The induction period is inversely related to the reactivity.

With the samples of calcium sulphate dihydrate which we have used the induction period of the derived hemihydrate has initially increased rapidly with the length of calcination and has finally become independent of it. With one particular sample calcined at 143° C. the induction period showed a pronounced maximum at 20 hr. (Fig. 1). With calcination of this sample at 203° C. the peak occurred at 5 hr., and at 106° C. there was no maximum in the induction period (that is, minimum in reactivity). There were corresponding but less-pronounced variations in k with length of calcination (Fig. 2). Similar changes in reactivity seem to occur when some samples of gypsum plaster are recalcined[1]. The induction periods for long times of calcination at 106° C. and 203° C. were considerably less than for calcination at 143° C. The magnitude of the increase in temperature during hydration showed that all the samples of calcined gypsum consisted essentially of calcium sulphate hemihydrate.

Variations in the reactivity of comparatively pure preparations of calcium sulphate hemihydrate, particularly when associated with sympathetic variations in k, are most likely to be due to differences in the density of seeding nuclei[1-3]. Large variations in surface area are reflected in the reactivity of calcium sulphate hemihydrate, particularly in the values of k[1]. However, Fig. 3 shows the surface area (error

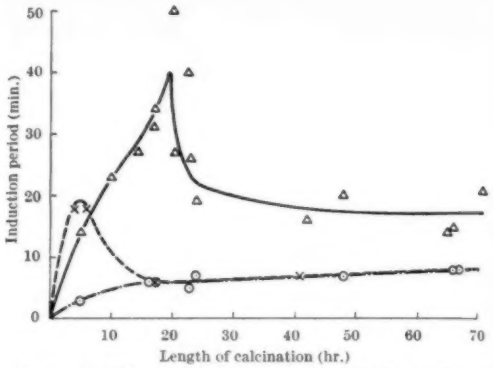

Fig. 1. Induction period plotted against period of calcination for samples prepared at 106° (○), 143° (△) and 206° C. (×)

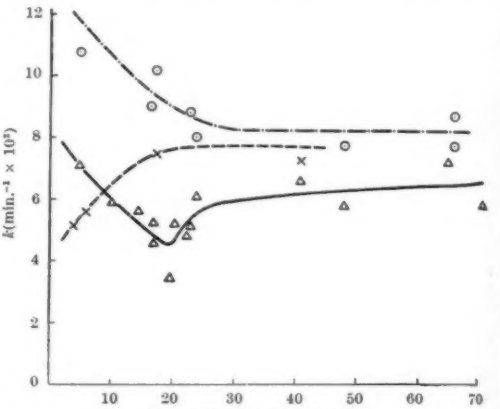

Fig. 2. k plotted against period of calcination for samples prepared at 106° (○), 143° (△) and 206° C. (×)

about ±0·3 m.²/gm.) determined by adsorption of ethanol for the samples prepared at 143° C.[1], and no pronounced minimum occurs corresponding to the minimum in reactivity (maximum in induction period), confirming that the observed effects are probably due to changes in density of nucleating sites in the system.

The variations in reactivity described here can be explained by assuming the occurrence of two reactions that involve nuclei for the growth of calcium sulphate dihydrate. The first is the decomposition of the dihydrate lattice, and appears to be the only one occurring at 106° C. (Fig. 1). As calcination continues the product becomes less and less reactive as seeding particles of residual dihydrate disappear. As the temperature of calcination is raised this reaction becomes more rapid. However, there intervenes a

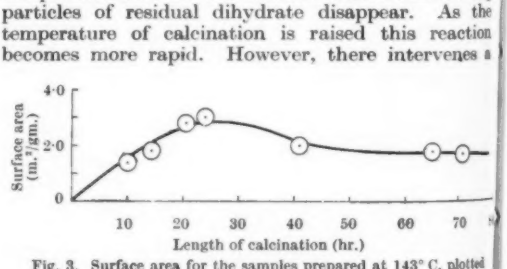

Fig. 3. Surface area for the samples prepared at 143° C. plotted against period of calcination

second reaction (presumably with a higher activation energy) that produces new nuclei so that at higher temperatures the reactivity first falls and then increases again. The nuclei produced by the second reaction are most unlikely to consist of fragments of dihydrate lattice, and the reaction probably involves some type of re-arrangement of the surfaces of the particles. The values of the surface area show no large variations, but the slight variations shown by the values for samples prepared at 143° C. may suggest a type of slow sintering after 20 hr. (Fig. 3). If the new sites favourable for nucleation of dihydrate do result from a re-arrangement of the surface of the grains of hemihydrate, they must be due to geometric features rather than to regions of strain.

The behaviour of some other samples where the peak is absent is due to the rates of these reactions, particularly the second, being so fast that the peak occurs so early that it is lost in the drop of reactivity caused by the first reaction. That an inhibitor which disappears at higher temperatures is not responsible for the effects described in this communication is indicated by the comparatively high reactivity of the samples prepared at 106° C.

This work forms part of a programme of research carried out within the Commonwealth Scientific and Industrial Research Organization and sponsored by the Colonial Sugar Refining Co., Ltd., and Australian Plaster Industries Pty., Ltd.

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BIOCHEMISTRY

Prevention of Ammonia Toxicity by Amino-acids concerned in the Biosynthesis of Urea

Relationships have been pointed out by several investigators[1] between high levels of blood ammonia and various clinical syndromes, for example, some psychoses, mental disturbances associated with heart failure, tumours, jaundices, hepatic encephalopathies, etc. In some cases a reduction in the level of ammonia has resulted in considerable amelioration of these pathological symptoms; the various procedures for this purpose are of considerable interest[1].

In 1956, Greenstein and co-workers[2] emphasized the antitoxic action of L-arginine in experimental hyperammonæmia produced by intraperitoneal injections of ammonium salts or high amounts of certain amino-acids. This work was followed by clinical studies, and some investigators[3] obtained beneficial results after administration of L-arginine; in all these cases the authors observed a striking reduction of blood ammonia which paralleled the alleviation of toxic symptomatology.

Different procedures for the same purpose have been employed with as yet indecisive results[1,4]. While the action of glutamic acid might be due to removal of the toxic ammonia via glutamine synthesis, the effect of L-arginine may involve a mobilization or enhancement of the Krebs–Henseleit urea-synthesizing cycle, resulting in a decrease of the ammonia-level; this hypothesis is supported by the observation of appreciable increases in the levels of blood urea[2,3].

Table 1. Effect of Prior Administration of Amino-acids on Rats given LD_{50} Doses of Ammonium Acetate

Protective compounds	Dose (m.moles/kgm.)	No. of animals	Survivors (per cent)	Incidence of convulsion (per cent)
Phosphate buffer	—	25	48	100
L-Ornithine + L-Aspartic acid	0·5	20	95	20
L-Ornithine + L-Aspartic acid	1·0	25	100	0
L-Ornithine + L-Aspartic acid	1·5	20	100	0
L-Ornithine	3·0	20	100	0
L-Aspartic acid	3·0	19	58	85
L-Aspartic acid + L-Ornithine	2·5	31	90	20
L-Lysine	3·0	10	50	100
L-Isoleucine	3·0	17	65	88
L-Isoleucine + L-Ornithine	2·5	27	63	80
L-Ornithine	0·5	32	93	43

The animals were Long–Evans male rats weighing about 250 gm., starved for a period of 12 hr. with water *ad libitum*. Both ammonium acetate and amino-acids dissolved in phosphate buffer (final *p*H 7·4), were administered intraperitoneally; the amino-acids 1 hr. before ammonium salt. Control animals were treated only with phosphate buffer before the ammonium acetate.

We have tested other substances concerned in the synthesis of urea for a similar effect. For this, some amino-acids, mainly L-ornithine and L-aspartic acid, have been tested in chronic carbon tetrachloride poisoning in rats[5]; their protective efficiency was evaluated on the bases of increase in weight and of the total lipid and glycogen content of the livers; the efficiency reaches 96 per cent if an ornithine–aspartic acid mixture (2·5 m.moles/kgm.) is used. The present communication describes the protective effects of prior administration of this mixture against experimental hyperammonæmia produced by intraperitoneal injection of LD_{50} quantities of ammonium acetate (8·2 ± 0·8 m.moles/kgm.) into rats.

The first set of experiments established the protective effects of some amino-acids against the mortality of rats suffering from ammonia intoxication (Table 1). The mixture of L-ornithine and L-aspartic acid, after intraperitoneal administration, gives optimum protection at levels of 2·0 m.moles/kgm. body-weight; at levels of 1·0 m.mole/kgm., 95 per cent of the animals survived. It is noteworthy that aspartic acid alone (3·0 m.moles/kgm.) has almost no effect, whereas the addition of ornithine in rather low concentration (0·5 m.mole/kgm.) suffices to raise the survival-rate to 90 per cent. We have also tested other amino-acids which are not involved in the synthesis of hepatic urea. L-Lysine alone (3·0 m.moles/kgm.) is completely inefficient, whereas L-isoleucine shows a protective effect slightly greater than that shown by aspartic acid alone; the addition of L-ornithine does not produce any increase of efficiency.

Levels of ammonia and urea in the blood of these animals were determined after various protective treatments (Table 2). In protected rats the rise of blood ammonia was quickly checked and, in animals treated with the ornithine–aspartic acid mixture (1·5 m.moles/kgm. of each), the values fell rapidly to normal levels. Simultaneously, the levels of blood-urea showed a marked increase.

These results suggest that ornithine and aspartic acid exhibit a specific detoxifying action toward

Table 2. Ammonia- and Urea-Levels in Blood of Rats protected against LD_{50} Doses of Ammonium Acetate by the Prior Administration of Mixtures of L-Ornithine and L-Aspartic Acid

No. of animals	Ammonium acetate	Protective mixture	Dose (m.moles/kgm.)	Blood ammonia (μmoles/ml.)		Plasma urea (μmoles/ml.)	
				Time after injection of ammonium-acetate (min.)			
				15	60	15	60
50	—	Phosphate buffer		0·76 ± 0·11*	0·76 ± 0·11	5·98 ± 0·73	5·98 ± 0·73
30	—	L-Ornithine + L-Aspartic acid	1·5 1·5	0·96 ± 0·12	0·71 ± 0·11	7·11 ± 0·54	7·81 ± 0·69
50	LD_{50}	Phosphate buffer		6·28 ± 0·33	2·59 ± 0·52	7·45 ± 0·76	9·87 ± 0·76
40	LD_{50}	L-Ornithine + L-Aspartic acid	0·5 0·5	3·77 ± 0·71	1·72 ± 0·72	7·66 ± 0·53	10·42 ± 1·34
50	LD_{50}	L-Ornithine + L-Aspartic acid	1·0 1·0	2·19 ± 0·39	1·39 ± 0·35	9·83 ± 0·90	12·76 ± 1·89
40	LD_{50}	L-Ornithine + L-Aspartic acid	1·5 1·5	2·38 ± 0·34	0·64 ± 0·39	10·53 ± 0·68	14·71 ± 1·81

The experimental conditions were as given in Table 1. Blood samples were drawn by cardiac puncture at the times indicated; the time of 15 min. was selected to coincide with the death of 50 per cent of the intoxicated animals. The survivors of this group were examined along with the other animals after 1 hr. Ammonia was determined in whole blood by Seligson's method (ref. 6); urea was determined in plasma by Kitamura's method (ref. 7).

* Mean values ± standard deviations.

blood ammonia. Their effect may be attributed to an enhancement of the Krebs–Henseleit mechanism of synthesis of urea.

The results of a more detailed investigation in progress will be published elsewhere.

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Chemistry of a Mucopolysaccharide produced by Guinea Pig Lymphocytes

The guinea pig lymphocyte has long been known to contain a special inclusion body. Such lymphocytes, called Kurloff cells after their discoverer, increase greatly in number in pregnancy and were shown by Ledingham[1] to be produced in both males and females by administration of œstrogens. Marshall and Swettenham[2], by histochemical methods, have shown that the inclusion body is composed of a mucoprotein–sulphated mucopolysaccharide complex. Further work on the chemistry of a mucopolysaccharide fraction by extraction of spleens of œstrogen-treated guinea pigs gave the following results:

(1) No linked amino-acids are present in the polysaccharide fraction; (2) the electrophoretic mobility in phosphate buffer at *p*H 7 on cellulose acetate is the same as chondroitin sulphate; (3) the bulk of the amino-sugar is composed of galactosamine (Gardell's method[3]); (4) the molar ratio of hexosamine, uronic acid and sulphate is the same as chondroitin sulphate *A* or *C*, that is, if hexosamine = 1 (sulphate = 1·29, uronic acid = 1·43 and sodium = 3·43); (5) the material is susceptible to testicular hyaluronidase. Some of the technical methods used in these estimations will be described later in a separate publication.

These results confirm the conclusion of Marshall and Swettenham[2] that the inclusion body of the Kurloff cell contains a material similar to the ground-substance of cartilage or connective tissue. The formation of large amounts of such material in the pregnant animal, however, remains of unknown significance and attempts to trace the fate of this polysaccharide in the pregnant guinea pig by labelling with sulphur-35 were unsuccessful. Furthermore, it is uncertain whether the formation of such material is peculiar to guinea pig lymphocytes or whether it is only the formation of the inclusion body which is unique to this species.

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Influence of Plasma Proteins in the Control of Mitosis-rates in Generating Liver

Many experiments have been done to elucidate mechanisms of control over growth-rates of liver, and in a comprehensive review of the regenerating liver Harkness[1] suggests that variations in the levels of plasma proteins, as shown by Glinos[2] and Gey[3], offer the most likely cause of changes in mitosis-rates after partial hepatectomy. Lately new evidence in favour of the removal of a normally present inhibitor has been offered from mouse skin[4] and from rat and guinea pig liver[5].

It was shown that adrenal corticosteroids could be the inhibitor normally present[5,6], and I now suggest control of mitoses in at least two ways: First, by a change in concentration of the plasma corticosteroids as shown by several workers[6–8] and to which normal cells are sensitive. Secondly, by a possible alteration of redox potential around each cell which has the effect of preventing corticosteroid influence on mitosis[5]. This effect has been shown by withdrawal of ascorbic acid from guinea pigs, but similar conditions could result from the output of oxidizing substances from damaged cells, such as tumours, or in wound healing where the levels of circulating corticosteroid may be high yet mitosis-rates are not inhibited.

In the experiments of Glinos[2] alterations are made in the concentrations of plasma proteins believed to

control mitosis; he demonstrates induction of mitosis in resting liver by dilution of plasma proteins and conversely a suppression of mitosis in regenerating liver by hæmo-concentration following water restriction.

Plasma proteins have been shown to carry corticosteroids[9,10], and it seems likely that the plasma protein influence is attributable, at least in part, to the associated corticosteroids; changes of protein-levels automatically altering the corticosteroid inhibition. To test this, Glinos's hæmo-concentration experiment has been repeated, but a comparison has been made between the degree of inhibition of liver regeneration in a dehydrated rat with intact adrenals and one with adrenals removed. If corticosteroids were the effective controllers of mitosis, adrenalectomy would allow a high rate of liver regeneration approximately double that found in rats with intact adrenals[5]. This has been demonstrated, even though plasma protein-levels have been maintained near those found in intact rats, which have no liver mitoses in a resting state.

Three groups of Wistar male rats weighing 200 gm. were compared. The first group of 60 rats were adrenalectomized and given food *ad lib.* but no water: 24 hr. later partial hepatectomy was done and the liver allowed to regenerate for 28 hr., when the rats were killed and a slice of the growing liver remnant taken for mitotic counting. The rats were bled by withdrawal of blood from the inferior vena cava and plasma protein estimated by the copper sulphate specific gravity drop method. Total time deprived of water was 52 hr.

The second group of 30 rats with intact adrenals were partially hepatectomized after deprivation of water for 24 hr. and were killed after 28-hr. liver regeneration, water being withheld also for a total of 52 hr. The third group of 10 rats with intact adrenals were given food and water *ad lib.* and partially hepatectomized. After 28 hr. liver regeneration the rats were killed and liver slices and blood collected for examination as in the preceding groups.

Mitotic counts of regenerated liver and plasma protein-levels are shown in Table 1 (mitoses per 1,000 cells).

Table 1. Plasma Protein and Mitotic Counts of Rat Liver regrowing 28 hr. after Partial Hepatectomy

	Group 1	Group 2	Group 3
	Dehydrated adrenalectomized rats	Dehydrated intact adrenals (gm.)	Water allowed, adrenals intact (gm.)
Plasma protein (gm.) (per cent)	7·9 ± 1·3	9·1 ± 1·1	5·9 ± 0·8
Mitotic rate	62 ± 8	16 ± 5	38 ± 6

(± *S.D.*)

In group 1 the adrenalectomized dehydrated rats showed a higher liver regeneration-rate almost double that of the intact hepatectomized rats having full access to water which formed the third group. In the former case plasma protein concentration has been maintained but absence of corticosteroids has removed all inhibition on mitosis.

Groups of dehydrated rats with intact adrenals show an inhibition of mitosis in the regenerating liver as reported by Glinos. Here plasma protein-levels have been maintained and in addition it is likely that corticosteroid output is high due to the stress of water deprivation.

The third group shows the plasma protein and mitotic rates in partially hepatectomized rats not deprived of water. Plasma protein-levels of intact rats average 8–9 gm. per cent.

It appears, therefore, that plasma proteins are only partly responsible for mitosis regulation in liver, and that they form a vehicle for the carriage of corticosteroids which are the effective control. The reasoning of Glinos is not invalidated by this extension as the homeostatic mechanism of maintenance of plasma proteins carries with it a built-in control, the cortical hormones, which only achieve an inhibiting effect on liver growth when this organ is of a size sufficient to maintain full plasma protein-levels and thereby a proper concentration of corticosteroids.

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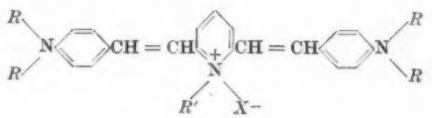
Pyridine Derivatives with Anthelmintic Activity

We should like to report a new series of chemical compounds which are effective against certain nematode parasites of mammals. Some of these compounds show a broad anthelmintic spectrum, being active against representatives of a number of families of nematodes.

These compounds can be illustrated by the general structural formula (I):



(I)

in which R can be, for example, alkyl or benzyl, or $N\begin{smallmatrix}R\\R\end{smallmatrix}$ can consist of a cyclic ring, such as pyrrolidine, piperidine, morpholine, etc., and R' can be alkyl or benzyl and X^- can be a suitable anion, most commonly iodide. These and numerous other structural variations have been prepared and tested, and details of syntheses will be published shortly.

A number of members of this series were found quite active against natural infections of the mouse pinworm, *Syphacia obvelata*, but two of the series (*A*: (I) $R = CH_3$, $R' = CH_3$, $X = I$; (I) $R,R = (CH_2)_4$, $R' = C_2H_5$, $X = I$) gave superior and almost equal activity with single doses of as low as 5 mgm. of base per kgm. of body-weight. Although *B* was slightly better against *Syphacia*, it was less effective than *A* against various nematodes of dogs and cats.

Compound *A* was tested extensively against nematodes of dogs and cats, and, to a limited extent, against nematodes of monkeys, using single doses of 25 mgm./kgm. or less for most parasites or multiple doses of 25 mgm./kgm. for whipworm infections. It exhibited a broad anthelmintic spectrum, showing good to excellent activity against natural infections of *Ancylostoma caninum*, *Toxocara canis* and *T. cati* in dogs and cats; *Uncinaria stenocephala*, *Toxascaris leonina* and *Trichuris vulpis* in dogs; and *Trichuris trichiura*, *Oesophagostomum apiostomum* and *Physaloptera* sp. in monkeys.

Inasmuch as rats, dogs and cats have tolerated single doses as large as 500 mgm. per kgm. of body-weight without evidence of ill effects, there is a wide margin of safety between the effective and toxic doses and, in addition, the compounds are tasteless and appear to have negligible emetic activity.

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Comparison of the Serum Protein Fractions of the Newly Hatched Chick with those of Adult Birds using Starch-gel Electrophoresis

It has been established by several investigators that serum protein components of chick embryo undergo qualitative as well as quantitative changes throughout the developmental period until the time of hatching[1–4]. The serum protein composition of the newly hatched chick, however, resembles that of the adult bird, as revealed by moving-boundary and paper electrophoresis. The technique of starch-gel electrophoresis suggested by Smithies[5] is promising in that it enables one to detect numerous fractions in the pattern of the serum proteins, particularly in the α- and β-globulin regions, which cannot be detected by the conventional method of Tiselius or paper electrophoresis. By using the starch-gel method, I have been able to demonstrate differences in the patterns of serum proteins of the day-old and week-old chicks when compared with pooled sera of one- and two-year-old roosters, as shown in Fig. 1. The electrophoresis was performed on a vertical tray[6] for 18 hr. using borate buffer of *p*H 8·6 and a field-strength of 6 V./cm. Repeated electrophoretic runs gave patterns similar to those shown in Fig. 1, for the four age groups. Twelve fractions could be distinguished on the patterns of the adult roosters (two pre-albumin components are not seen on the photographs). The same number of fractions could also be seen on the electrophoretic patterns of the young chicks. The mobility and the number of fractions of α-globulins of the newly hatched chicks are distinctly different from those of adult birds. The fraction *b* of the serum of a day-old chick does not seem to be present in the serum of an adult bird, and of fraction *d* only traces are observed. Very little γ-globulin is present in day-old and week-old chicks. A distinct narrow band which is immediately behind the albumin (a post-albumin ?) in the adult bird cannot be seen in the young chicks.

Fig. 1. Starch-gel electrophoresis of the sera of chicken at various ages. I, Day-old chick; II, week-old chick; III, one-year-old rooster; IV, two-year-old rooster

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Cholesterol in the Tapetum Lucidum of the Eye of the Opossum, *Didelphis virginiana*

The eye of the opossum *Didelphis virginiana* has a tapetum formed from the retinal pigment epithelium[1,2]. Investigation of the eyes of three specimens of *Didelphis virginiana* and of one specimen of the crab-eating opossum obtained through the kindness of Dr. S. Halbert, College of Physicians and Surgeons, New York, and of Dr. Osman Hill, Zoological Gardens, London, has shown that the tapetum of these animals is rich in cholesterol, cholesterol esters and lipids and it is tentatively suggested that these form the reflecting material *in vivo*.

The tapetum of the opossum, viewed ophthalmoscopically immediately after death, appeared as a triangular dead-white area. Dissection of the tapetal area was unsatisfactory, both in unfixed and in eyes fixed with formol, as it was difficult to separate it from overlying retina and underlying choroid. The roughly separated tapetum was dried, extracted with chloroform–methanol 1 : 1 (v/v), and both extract and residue were examined. Microscopical examination of the residual material showed that birefringence of the tapetum had been removed. The chloroform–methanol extract was dried and the residue extracted with acetone. The acetone-soluble fraction consisted largely of cholesterol as judged by estimation[3]. Paper partition chromatography[4], using 95 per cent methanol as solvent, showed that cholesterol esters and/or fat were present as well as free cholesterol. In this system cholesterol esters and fat remain at the origin and cholesterol has R_F 0·50. Markers of cholesterol, cholesterol oleate and cholesterol stearate were run in parallel. To determine whether the material in the tapetum which remained at the origin on the chromatogram contained cholesterol esters or was entirely fat, an aliquot was saponified and re-chromatographed alongside the original material. The spot at the origin disappeared on saponification and the spot corresponding to cholesterol became more intense and larger, which suggests that cholesterol esters are present.

One eye was examined histologically. After fixation with formol, the eye was opened and the lens, which is large and spherical, was removed. The back part of the eye was then embedded in gelatin and

frozen-sections were cut and floated on gelatin-coated slides. When the Lieberman–Burchardt reagent was dropped on the sections, a strong positive cholesterol reaction developed in the area of the tapetum, and a weaker one was given by the optic nerve. Retina and choroid were negative. Staining with sudan III showed that the tapetal area was also rich in lipid. A few lipid globules were present in the choroid and orbital tissues.

These experiments showed that the opossum tapetum contained cholesterol, cholesterol esters and lipid, and that when these were extracted the birefringence of the original material was lost. An attempt was therefore made to estimate the total cholesterol of the different parts, dissected from a single eye of *Didelphis virgiana* (Table 1).

Table 1. CHOLESTEROL CONTENT OF DIFFERENT PARTS OF THE EYE OF *Didelphis virginiana*

Part of eye	Dry weight (mgm.)	Total cholesterol (per cent dry weight)
Sclera	84	0·4
Choroid	7·3	5·8
Retina	2·8	18·2
Tapetum	1·0	50·0

Table 1 shows that the dissected tapetum is richer in cholesterol than the other parts. Chromatography of this material before and after saponification showed that the cholesterol was largely esterified. It is probable that most of the cholesterol found in the dissected retina and choroid comes from contaminating tapetal tissue. Cattle retina contains only 2·0 per cent cholesterol and cattle choroid 0·80 per cent[5].

Roughly dissected tapetum from the unfixed eye was birefringent but crystals were not clearly detected. Crystals (Fig. 1) could be brushed off the tapetum of the eye fixed with formol and similar crystals were deposited from the chloroform–methanol and subsequent acetone extract of tapetal material. The appearance of the tapetum seen ophthalmoscopically or in the opened eye is chalk-white, without the brilliance which one sees from the crystals of guanine in the argentea and tapetum of fish. There is no iridescence.

Different animals solve the optical problem of providing a mirror backing to the retina in widely different chemical ways. The crystalline tapetum in fish and reptiles is made of guanine[6], in carnivores of zinc-cysteine[7,8], in lemurs of riboflavin[9], and in the opossum of cholesterol esters and lipid. In fish, guanine is also laid down in the scales, and in lemurs Dr. Osman Hill (personal communication) has detected large amounts of a yellow water-soluble pigment in the skin, which is probably riboflavin. Whether zinc-cysteine occurs in other tissues of carnivores and whether there is particular concentration of cholesterol and lipid in other organs in the opossum are not known.

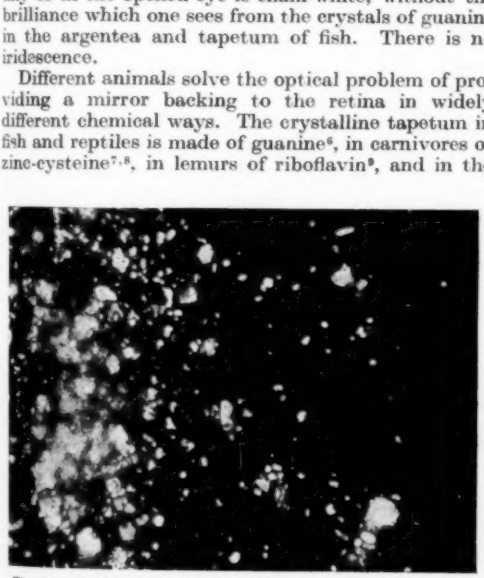

Fig. 1. Crystals brushed off tapetum of *Didelphis virginiana*

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PHYSIOLOGY

Localization of the 'Sugar Pump' in the Intestinal Epithelium

GLUCOSE is actively transported across the intestinal epithelium from the lumen of the gut into the blood *in vivo* or into the fluid surrounding the serosal mesothelium *in vitro*. Because part of the glucose is metabolized during transport, it is not possible to determine the concentration of the unaltered sugar in the various layers of the intestine. However, 3-O-methylglucose, though transported by the same mechanism which acts on glucose[1], is not metabolized in animal tissue[2,3]. The synthesis of 3-O-$^{14}CH_3$-glucose in this laboratory[3] offered a method of following with reasonable accuracy the route of this sugar during transport.

Using surviving preparations of the small intestine of frogs (*Rana pipiens*), the following experimental procedure was employed[4]. 3-O-methylglucose (mixed with 3-O-$^{14}CH_3$-glucose) was dissolved in a Ringer's solution isosmotic with frog's blood and containing sulphate instead of chloride in order to minimize net water transport. The frogs were pithed, the abdomen was opened and the small intestine was ligated at both ends and injected with 1 ml. of the 3-methylglucose solution. The intestine was then excised and transferred to a flask containing 2 ml. of the solution identical with that which was injected into the lumen of the gut. The preparation was briefly gassed with a mixture or 95 per cent oxygen plus 5 per cent carbon dioxide and shaken in a 30° water-bath for 6 hr. Then the intestine was removed, carefully blotted on the outside and emptied of its contents. Thereafter, the intestine was split open, the mucosal surface gently blotted with a piece of cotton and the mucosa scraped off, using a coverglass. The mucosal scrapings were weighed, placed in a test-tube containing a known volume of hot water and refluxed in a hot water-bath for 10 min. The remaining portion of the intestinal tissue ('submucosal tissue') was similarly weighed and extracted with boiling distilled water.

Thus, at the end of the experiment, there were four different solutions : the contents of the lumen

Table 1. Distribution of 3-O-$^{14}CH_3$-Glucose in the Small Intestine of the Frog

Exp. No.	Initial 3-methyl-glucose m.mol./litre	Initial 3-methyl-glucose 10^3 counts/min./ml.	Distribution of radioactivity at 6 hr. 10^3 counts/min./ml. Inside	Mucosal	Sub-mucosal	Outside
1	10	20·00	3·96	19·41	21·52	24·29
2	10	20·00	7·68	19·41	24·14	22·08
3	10	20·00	6·14	21·48	23·00	24·96
		Average	5·79	20·10	22·89	23·78
4	2·5	20·00	6·63	17·10	22·47	21·54
5	2·5	20·00	9·93	14·95	18·22	17·03
6	2·5	20·00	4·85	18·19	19·39	16·98
		Average	7·14	16·75	20·03	18·52

('inside fluid'), the extract of the mucosal scraping, the extract of the submucosal tissue, and the 'outside fluid' bathing the serosal mesothelium. From each solution samples were plated on to planchets and the radioactivity was counted in a windowless flow counter. The results were corrected for self-absorption. The water content of the tissue samples was calculated on the basis of an average value obtained in a series of determinations on both mucosal scrapings and submucosal tissues. The activity was expressed as counts/min./ml. of tissue water and of inside and outside fluids.

The results are shown in Table 1. The two series of experiments differ only in the initial concentration of 3-methylglucose; the amount of the tracer was the same in both series. As the principal anion was sulphate, which in turn kept the net movement of water across the intestine to a minimum, the change in concentration of the tracer in any compartment reflects the actual movement of 3-methylglucose regardless of its original concentration. It is evident from the results in Table 1 that during the experiment the concentration of 3-methylglucose decreased considerably in the lumen of the intestine, and that the concentration of 3-methylglucose in the water of the mucosal epithelial cells increased to a level two to three times higher than that in the lumen of the gut. The differences in concentration of 3-methylglucose between the other layers of the intestine and the serosal compartment are not so striking, and it is questionable if they were of any significance.

These results would suggest the following hypothesis: membrane facing the lumen of the intestinal epithelium is relatively impermeable to the sugar, and it is the site of the active transporting 'pump'. This would allow the sugar to be transported into the cell against a gradient and at the same time prevent a retrograde movement out of the cell into the lumen. The serosal-facing membrane of the epithelial cell is relatively permeable to sugar and so are the other tissues lying between the epithelial cells and the free serosal compartment. Thus, no appreciable concentration changes occur across these barriers.

These experiments do not indicate directly whether or not 3-methylglucose is present in the mucosal epithelial cells in the free or bound form. The concentration of 3-methylglucose is almost identical in the epithelial layer and in the subepithelial tissue as well as in the serosal compartment. If part of the sugar were bound in the mucosal cells, one would have to assume that a second concentration gradient of free sugar across the serosal-facing membrane occurred.

Attempts to demonstrate the presence of protein-bound 3-methylglucose in the extracts of intestinal mucosal epithelium were not successful. In these experiments the mucosal scrapings of 3-O-$^{14}CH_3$-glucose-absorbing guts were extracted with either cold saline or cold 0·6 *M* potassium chloride. Both extracts were centrifuged at 100,000*g* for 8 hr. More than 90 per cent of the total nitrogen of the extract was in the sediment, but the radioactivity in the supernatant did not change.

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Increase in Elastin Content of the Human Aorta during Growth

It is generally assumed that the elastic properties of the gross arteries, such as the aorta, are essentially due to elastic fibres of the arterial wall. However, there are few reports of quantitative studies of elastin in aorta; and often statistical proof of significance is omitted and conflicting statements have resulted. In particular, more detailed investigations on the elastin behaviour of the human aorta during growth are required.

Smith *et al.*[1] referred to increases in elastic tissues of the media with ageing, whereas no variation of the elastin content was found by Hass[2] or by Lansing *et al.*[3]. In agreement with the considerable decrease of elasticity of the aorta observed in ageing, Myers and Lang[4], Faber and Møller-Hou[5] and Bertolin[6] reported elastin as being highest in early life and gradually declining in the last decades of life. Kraemer and Miller[7], however, the only authors who reported statistical analysis of their own results, demonstrated an absence of changes with age in the percentage of aortic elastin.

All methods hitherto used in the determination of elastin consist basically of destroying materials other than elastin and gravimetric determination of the remaining insoluble substance. The availability of a new and specific method that permits the direct colorimetric estimation of elastin without destructive processes led me to review the behaviour of elastin in human aorta during growth and ageing.

This preliminary communication discusses the results of work on 16 normal young subjects up to 20 years old.

The direct method previously described by me[8], later further improved[9], has been used. The sample of tissue is treated with orcein. All free stain is removed and the stained orcein-elastin compound hydrolysed with elastase (pancreatin). Orcein-elastin, which is brought in this way into solution, is then estimated photometrically.

Fresh specimens were removed at the time of autopsy from the aortæ of apparently normal male subjects between 0 and 20 years of age who died from trauma. Specimens free from atherosclerotic lesions were always taken from the inferior part of thoracic aorta, stripped of adventitia and treated

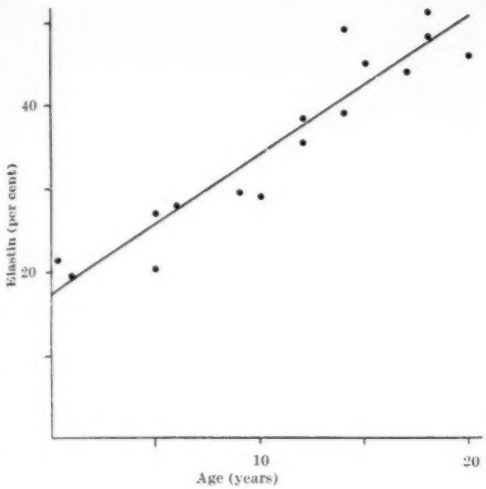

Fig. 1

as already described by me. 30 mgm. of the dry triturated matter were taken for analysis.

In Fig. 1 the results of analyses are expressed as percentage of elastin in the dry weight of the sample and plotted against age at time of death. Statistical analysis of this graph shows a highly significant increase in elastin content of the human aorta with age between 0 and 20 years (correlation coefficient: $r = 0{\cdot}877$; Student's $t = 6{\cdot}81$; $P < 0{\cdot}001$). The equation of the regression line is $y = 17{\cdot}5 + 1{\cdot}65\,x$.

Further investigations in progress in this laboratory seem, on the other hand, to demonstrate a significant decrease of elastin content in the aorta after 20 years until old age.

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Lack of Effect of Coenzyme Q_{10} on Coumarin-induced Hypoprothrombinæmia

Several coumarin and indanedione drugs are capable of inducing hypoprothrombinæmia, presumably by interfering with the cofactor role of vitamin K in prothrombin synthesis. This role of vitamin K, probably involving electron transport, can be competitively inhibited by coumarins, and the inhibition competitively overcome by large doses of vitamin K (ref. 1).

Recently[2] another group of quinones, the coenzymes Q, has been shown to be involved in electron transport. Numerous similarities of coenzymes Q to the vitamin K group[3] have led to the following comparison of vitamin K_1 and coenzyme Q_{10} as regards inhibition of the prothrombin response to a coumarin anticoagulant.

Table 1. Effect of Coenzyme Q_{10} on Prothrombin Response to Acenocoumarin in Normal Guinea Pig*

	Prothrombin time (sec.)	
	Range	Average
Q_{10} vehicle (1 or 2 c.c.)	31–34	33
Q_{10} (10 or 20 mgm.)	31–36	34
Acenocoumarin (1 dose) 4·5 mgm. in 1 c.c. H_2O	49–62	55
Acenocoumarin (1 dose) + Q_{10} 10 mgm.	53–130	79
Acenocoumarin (2 doses 24 hr. apart) each 4·5 mgm.	99–>180	>149
Acenocoumarin (2 doses 24 hr. apart) + Q_{10} 10 mgm.	78–177	134
Acenocoumarin (2 doses 24 hr. apart) + vitamin K_1 ('Mephyton') 10 mgm.	35–50	43

* All drugs were given intraperitoneally. Each group consists of at least four animals.

Guinea pigs were treated with sodium acenocoumarin, vitamin K_1, coenzyme Q_{10} (10 mgm./c.c.) in solvent vehicle (consisting of 'Emulphor' and dimethylacetamide, and the co-enzyme Q_{10} kindly provided by Dr. Karl Folkers of Merck, Sharp and Dohme, Rahway, New Jersey) and solvent vehicle as indicated in Table 1. Blood specimens were obtained 24 hr. after the last dosage, and the prothrombin time estimated by techniques previously reported[4]. The relatively long normal prothrombin time by the method employed is characteristic of the species. The results in Table 1 demonstrate that coenzyme Q_{10} has no vitamin K-like protective effect against acenocoumarin-induced hypoprothrombinæmia under the experimental conditions used.

Table 2. Effect of Coenzyme Q_{10} on Response to Acenocoumarin in Acutely Starved Guinea Pigs

	Prothrombin time (sec.)	
	Range	Average
Acenocoumarin 4·5 mgm. + 1 c.c. saline	40–55	48
Acenocoumarin 4·5 mgm. + coenzyme Q_{10} 10 mgm.	60–75	68
Acenocoumarin 4·5 mgm. + 1 c.c. vehicle	50–87	63

To test this observation further, the experiment was repeated in 12 animals deprived of food the day prior to and after dosage. The results are presented in Table 2. It is evident that coenzyme Q_{10} had no protective effect.

It is concluded that in the guinea pig, exogenous coenzyme Q_{10} has no vitamin K-like antagonistic action against the hypoprothrombinæmia induced by acenocoumarin.

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Pinnal Anaphylaxis: an Additional Anaphylactic Site

In some respects the mouse is a desirable experimental animal for immunological studies. Because of its small size and high reproductivity it can be used in statistically meaningful numbers. Availability of highly inbred strains minimizes variation in individual response and makes it possible to select strains of maximal sensitivity[1].

In other respects the mouse has certain drawbacks. One is its relative refractoriness to anaphylactic challenge. Another is the limited body-area available for local anaphylaxis.

Ovary[2] demonstrated the possibility of producing passive cutaneous anaphylaxis in the skin of the back of the mouse. He found that about 100 times as much rabbit antibody is necessary to induce local sensitization of the mouse skin as compared with the guinea pig. With high concentrations of reagents he was effectively limited to two injection sites, one on each side. His intravenous challenging dose of antigen was 1·2-mgm. hen ovalbumin.

The present investigation was undertaken with the objective of increasing the number of available sites for local anaphylactic challenge in the mouse and, perhaps, introducing more sensitive sites than the skin of the back. The ear was selected for several reasons. It is a translucent structure, in which collection of dye released by local anaphylactic reaction would be readily apparent. Though preferential inducement of capillary permeability in the ear, as compared with the skin of the back, is less marked in the mouse than in the rat and guinea pig, Feldberg and Miles[3] did find a distinctly greater response to 48/80 over the head, shoulders and limbs than elsewhere on the mouse skin. Finally, as a paired structure, one ear can, if desired, serve as a control to the reaction in the other. This initial communication reports primarily on the production of passive anaphylaxis in the ear of the mouse, using a partially purified duck ovalbumin and the rabbit antiserum to it.

The skin over the ear is very fine, and it is impracticable to carry out intradermal injection. Therefore, it was decided to instil the sensitizing serum subcutaneously. The mouse was lightly anæsthetized with ether. Using 26 gauge × ½-in. needles, a tenfold dilution of the antiserum was injected between the skin and cartilage on the outer aspect of one ear, while saline was injected similarly in the other. Volume was controlled by sight, a small bleb being raised. This approximates to 0·025 ml. of fluid per site.

At intervals varying from 30 min. to 24 hr. after sensitization the mice were injected intravenously in a tail vein with 0·25 ml. of a solution containing 1·25 mgm. of 'Pontamine' sky blue and 0·1 mgm. of the duck ovalbumin. Control mice were challenged with dye alone. A positive reaction, as exhibited by the local extravasation of blue dye in the ear, generally appeared within 10 min. and was complete after 30 min. The saline-injected control ears in antigen-challenged mice were uncoloured (apart from an occasional pin-prick of blue at the point of passage of the needle through the skin), as were both ears of the mice challenged with dye alone. Fig. 1 shows the complete results of a single experiment: no selection or elimination has been made. Positive reactions have been obtained when antigen challenge has been carried out as early as 30 min. and as long as 24 hr. after passive sensitization of the ear, 2–3 hr. being the optimal time for production of uniformly strong reactions.

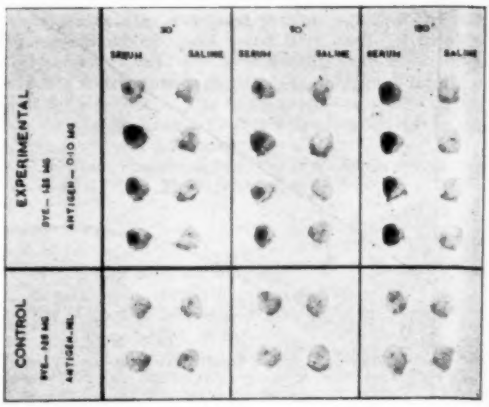

Fig. 1. Passive pinnal anaphylaxis in the mouse. Ears seen from inner aspect

The term pinnal anaphylaxis seems most appropriate to the phenomenon as the entire thickness of the pinna appears to be permeated with the dye; the skin on both the inner and outer aspects of the ear, the subcutaneous spaces and the cartilage.

Passive pinnal anaphylaxis has also been produced in intravenously blued mice on subcutaneous challenge with as little as 0·5 mgm. of duck ovalbumin. It has also been demonstrated in the guinea pig both on intravenous and subcutaneous challenge with duck ovalbumin and grass pollen extracts, using appropriate antisera. Quantitative studies, active pinnal anaphylaxis and other extensions and applications of the phenomenon are under investigation.

I wish to thank Miss Janet Dewdney for technical assistance, and Douglas F. Lawson for photographing the results.

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Cytochrome *c* in the Heart of the Turtle

In a thesis on the anaerobic metabolism and mechanical efficiency of the heart of the turtle, by R. Blake Reeves[1], there is evidence that this heart is able to work efficiently for any length of time without access to oxygen (nitrogen anoxia). In view of this remarkable quality we found it interesting to investigate the content of cytochrome *c* in the heart of the turtle (*Testudo graeca*). To our knowledge no such data are available in the literature[2–4], although reports on the cytochrome *c* content of the heart of the frog have been published by Keilin[5].

Six turtles were killed by ether and the hearts were weighed (3·5 gm.). The cytochrome *c* was extracted according to a method described by one of us (G. B.[4]); instead of 'Amberlite *XE*-64', however, 'Duolite *CS*-101' was used in the present investigation.

On analysis of the final eluate (reduced state) there was a typical cytochrome *c* spectrum with absorption maxima at 417, 521 and 550 mμ. The ratio $\frac{550\ m\mu\ red.}{280\ m\mu\ ox.}$ = 0·3 was very low compared with those of previous investigations[5]. Using the value of $E^{1\ cm.}_{1\%}$ 550 mμ = 22·7 for crystalline bovine cytochrome *c*[6], the concentration of cytochrome *c* in turtle heart muscle was 2·7 mgm./100 gm. wet weight, which is about the same content as that of heart muscle of the frog[3].

We have found it of some interest to report this observation in connexion with the concept of the electron transport particle advanced by Green[7]. Further investigations on respiratory enzymes in poikilotherms are in progress.

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RADIOBIOLOGY

Failure of a Dog to discriminate between Strontium-90 and Calcium given Orally

THE ability of a mammal to discriminate against strontium-90 has been the subject of intensive study in recent years. A recent summary of the results of several experiments using a variety of species and experimental approaches indicated that bone discrimination against strontium-90 in favour of calcium can vary from 2 to 4 (ref. 1). This means that the ratio of strontium-90 to calcium in bone as compared with the diet, referred to as the observed ratio, ranges from about 0·25 to 0·50 (ref. 2). These observations have led to the general belief that the mammal always discriminates against strontium-90 deposition to some significant extent.

There are a few reports of a lack of discrimination against orally administered strontium-90. Comar observed a ratio of 1·0 in cattle given a single oral dose of strontium-90 or calcium-45 (ref. 3). No work has been reported in which large animals received strontium-90 orally for long periods of time. However, rats were fed throughout their lifetime on a constant strontium-90/calcium diet and exhibited a ratio of 0·28 (ref. 4).

This investigation is concerned with beagles which were fed a constant diet of strontium-90 (1·8 μc. strontium-90/gm. dietary calcium) from the beginning of fœtal ossification (20–30 days after mating) until the pups reached early adulthood (540 days of age). It had previously been noted that the observed ratio between the fœtus and the dam's diet was 0·28, but that the growing pup, when maintained on the same diet, appeared to exhibit a ratio of about 1·0 (ref. 5). One of these pups was maintained on this same diet, first, through the dam during both the last half of gestation and the period of lactation, and secondly, from weaning until 18 months of age, at which time it was killed. The diet fed the dam and her litter was composed of 60 per cent dry kibbled ration to which were added 20 per cent beef and 20 per cent (by weight) water, including the strontium-90 dose. Dietary calcium was essentially all in the dry ration, which, by the classic permanganate method, was found to contain 3·3 per cent calcium by weight. Strontium-90 was mixed into the complete ration so that there were 1·8 μc. strontium-90 per 1·0 gm. calcium at the date of killing. The diet was fed *ad libitum* throughout the experiment to ensure a physiological approach.

The beagle grew normally and was in excellent condition throughout the experiment. It exhibited no lesions either radiographically or on gross and sub-gross examination of the organ systems. The humerus, a rib, and one half of the mandible including teeth were removed, stripped of their muscular attachments, and stored in acetone. The bones were dried later and the humerus was sawn into the eight pieces which are specified in Table 1. Each sample was ashed and then analysed for strontium-90 and calcium. The β-detector used was a well-type plastic scintillator; details of the strontium-90 measurement have been reported in ref. 6. Calcium in the bone samples was determined complexometrically with ethylenediamine tetraacetate (EDTA), which contained a small amount of magnesium chloride, at $pH > 10$ using eriochrome black *T* as the indicator. Magnesium in these samples comprised about 1 per cent of the calcium on a molar basis. The results are given in Table 1.

Table 1. CONCENTRATION OF STRONTIUM-90 IN BONE FOLLOWING CONTINUOUS EXPOSURE BEGINNING *in utero* TO 18 MONTHS OF AGE

Samples	gm. calcium	gm. calcium/gm. ash	μc. strontium-90*	μc. strontium-90*/gm. calcium
Proximal epiphysis	0·260	0·391	0·50	1·9
Proximal epiphyseal plate	0·724	0·398	1·2	1·7
Proximal metaphysis	0·744	0·392	1·3	1·8
Proximal diaphysis	0·541	0·393	1·0	1·8
Mid-diaphysis	0·475	0·388	0·97	2·0
Distal diaphysis and metaphysis	0·476	0·388	0·93	1·9
Distal epiphyseal plate	0·689	0·389	1·3	1·9
Distal epiphysis	0·207	0·392	0·43	2·1
Entire humerus	4·12	0·390	7·7	1·9
Rib	0·492	0·386	0·94	1·9
Left mandible with teeth	3·68	0·381	7·4	2·0
Diet				1·8

* At date of killing.

We conclude then that the integrated effect of an exposure of a beagle to an essentially constant dietary strontium-90/calcium level which began *in utero* and continued to young adulthood is that the relative strontium-90/calcium content of the bone is the same as that of the dietary environment. Furthermore, we can compare the distribution along the length of the humerus and in different bones, and the variation is not significant at the 95 per cent confidence-level. Thus the macroscopic distribution in bone does not vary from bone to bone or from trabecular to cortical bone.

Our observation of a value of unity for the ratio in contrast with other reports of lower values serves to emphasize the complications in measuring the over-all effect of many variables, namely, species, age, nutrition, duration of exposure. Finally, we recognize that 1·8 μc. strontium-90/gm. calcium is quite a high level, and, although the skeleton of the dog appeared normal in all respects, our observations do not preclude the possibility of microscopic alterations in bone growth and remodelling processes which may have affected the results.

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Carbon-14 from Nuclear Explosions as a Short-term Dating System: Use to determine the Origin of Heartwood

The concentration of carbon-14 dioxide in the atmosphere has been increasing since 1954 in a manner shown (for the southern hemisphere) in Fig. 1 (refs. 1,2). This results from carbon-14, produced in nuclear explosions and carried up into the stratosphere by the associated heat, slowly diffusing into the atmosphere. This phenomenon can be used as a short-term dating system. Its use to study the mechanism of heartwood formation is described here.

It is not known if the heartwood extractives present in the heartwood of a tree are the result of 'waste' products of metabolism of the tree at the time of formation of the heartwood, or whether they represent material which has always been present in the sapwood ring and which is converted into heartwood extractives when the wood is converted from sapwood into heartwood. In general, the first hypothesis is favoured. To examine this point, the three outer heartwood rings (the 12th, 13th and 14th rings counted from the outside) of a *Pinus radiata* (total number of rings, 24) felled on August 4, 1959, in the Kaingaroa Forest, New Zealand, were taken and the heartwood extractives removed by acetone and ethyl acetate extraction in a Soxhlet apparatus.

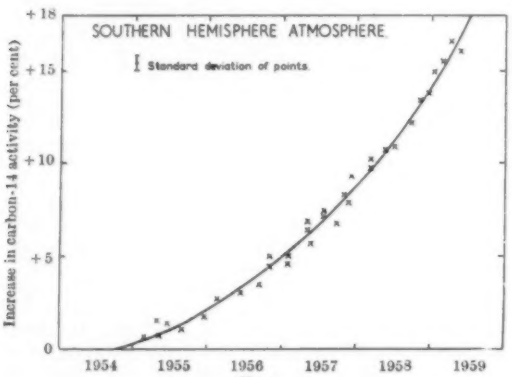

Fig. 1. Increase of carbon-14 in southern hemisphere atmosphere (after Ferguson, ref. 1)

Cellulose was then extracted from the residue. The cellulose and heartwood extractives were then converted to carbon dioxide and counted in the Institute of Nuclear Science low-level carbon-14 counter. The results (all corrected to the same carbon-12/carbon-13 ratio to allow for any isotope effect in the tree or in the experimental manipulation) are given in Table 1.

Table 1. Activity of Sample in Counts per min. for a 1-atm Counter Filling

All samples corrected to same carbon-12/carbon-13 ratio to correct for isotope effect in the metabolism of the tree and in laboratory manipulation. σ = Standard deviation of the counting

	Cellulose	Heartwood extractives	For comparison, the atmosphere at the time of formation of heartwood is given
Outer ring of heartwood	39·5 σ = 0·17	39·7 σ = 0·17	47·2
First inside ring	39·7 σ = 0·17	39·8 σ = 0·17	44·8
Second inside ring	39·4 σ = 0·17	39·1 σ = 0·23	42·6

It is seen that for a given ring of heartwood, the extractives correspond in activity to the cellulose and not to the atmosphere at the time of their conversion from sapwood to heartwood. The activity of the cellulose corresponds to the activity of the atmosphere when it was the outer ring of the tree (before nuclear explosions).

More specifically, it might be said that (at the 99 per cent statistical confidence-level) less than 6 per cent of the carbon in the heartwood extractives in the outer ring of the heartwood comes from the atmosphere in the year of its conversion from sapwood to heartwood. This might suggest that the sapwood of a pine tree always contains the material which will be converted to the heartwood extractives when that portion of the tree is converted from sapwood to heartwood. This raises the interesting theoretical possibility of artificially inducing the formation of heartwood.

It is hoped to publish later a detailed account of this and related work.

It should be pointed out that this phenomenon of increase of carbon-14 in the atmosphere provides a short-term dating system of great value for biological and other problems[2]. Due to exchange with the oceans, this unique situation will not last for more than a few years[2]. Thus, full use should be made of this effect by as many workers as possible in the immediate future.

The experimental work described in this communication was carried out when I was on the staff of the Institute of Nuclear Science, Lower Hutt, New Zealand. I thank the Forest Research Institute, Rotorua, for supplying the samples.

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Removal of Internally Deposited Radionuclides by Triethylenetetraamine-hexaacetic Acid

INCREASING the number of donor groups in chelating agents can give rise to the formation of more stable chelates[1-3] and, therefore, to a higher mobilization of internally deposited trivalent and other metal ions of higher valency. This has been demonstrated with ethylenediamine - NNN'N' - tetraacetic acid (EDTA) and diethylenetriamine-NNN'N'N''-pentaacetic acid (DTPA)[4,5], that is, with chelating agents with 6 and 8 donor groups respectively. For this reason extension of this work to the 10-dentate triethylenetetraamine - NNN'N'N''N'''- hexaacetic acid (TTHA) seemed to be worth while.

Carrier-free cerium-144, yttrium-91 or strontium-85 was injected intravenously in rats 7–9 weeks old averaging 165 gm. in weight. The chelating agents (by courtesy of J. R. Geigy, S.A., Basle) were given immediately or at different time-intervals after the injection of the nuclide. As shown in Table 1, TTHA reduces the radiocerium content of the skeleton more effectively than DTPA, when given simultaneously or immediately after the administration of the nuclide. In the liver, however, under the same conditions, DTPA is more effective than TTHA. The comparative higher effectiveness of DTPA in mobilizing deposited radiocerium from the liver becomes still more pronounced if the chelating agent is administered after longer time intervals, whereas the superiority of TTHA over DTPA in the removal of radiocerium from the skeleton vanished. Analogous results have been obtained for radioyttrium (Table 2); but neither TTHA nor DTPA had any effect on the mobilization of radiostrontium.

The causes for the observed differences in the behaviour of these two chelating agents are not yet understood. It may be assumed tentatively that TTHA forms to some extent polynuclear colloidal chelates which may be metabolized differently from the smaller molecules of the 1 : 1-DTPA chelates. The greater effectiveness of TTHA as compared with DTPA in the removal from the skeleton—the critical organ for most radionuclides—of radiocerium and radioyttrium can be of therapeutical value in certain cases and justifies further investigations on other radionuclides, especially plutonium.

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Incorporation of Phosphorus-32 into Urinary Concretions forming on Zinc Implants in Rat Bladders

IT has been proposed that labelling with radioisotopes might be useful in studying the rate of formation of urinary calculi in experimental animals as a function of environmental conditions. The proposal was based on the fact that in siliceous urinary stones of bovine origin, which contain traces of phosphorus and calcium, diffusion and subsequent exchange of phosphorus-32 and calcium-45 with the non-radioactive counterparts of the stone material do not readily occur across the mucoprotein laminations[1]. It was felt that if stone formation occurs layer by layer, short-term massive administration of phosphorus-32 to an experimental animal forming a laminated calculus would result in incorporation of the radionuclide into a single or limited number of laminations, and that subsequent exchange into other laminations would be negligible. Further, if administration of phosphorus-32 were then stopped, and exchange from other parts of the body did not cause significant levels of phosphorus-32 in urine, subsequent laminations would be relatively free of label. Therefore periods of exposure to various environmental conditions could be alternated with periods of administration of isotope, and the amount of material deposited between the laminations

Table 1. INFLUENCE OF DTPA AND TTHA ON THE DISTRIBUTION OF CERIUM-144. THE ANIMALS WERE KILLED 3–12 DAYS AFTER THE INTRAPERITONEAL ADMINISTRATION OF THE CALCIUM CHELATES. THE DOSAGE, IF NOT OTHERWISE STATED, WAS 250 μMOLES/RAT

Treatment		No. of animals	Per cent of control Mean and fiducial limits ($P = 0.05$) Liver	Kidneys	Skeleton
100 DTPA	simult. (intravenous)	10	0·11 (0·08– 0·14)	14 (9·5– 21)	1·3 (0·9 – 1·8)
" TTHA	simult. (intravenous)	10	0·23 (0·17– 0·31)	14 (9·1– 21)	0·45 (0·33– 0·62)
DTPA	after 2 min.	28	1·00 (0·78– 1·30)	19 (16·0– 23)	10·00 (9·00–12·00)
TTHA	after 2 min.	27	1·50 (1·30– 1·70)	16 (14·0– 19)	5·20 (4·50– 6·10)
DTPA	after 24 hr.	22	21·00 (19·00–24·00)	54 (43·0– 68)	72·00 (64·00–80·00)
TTHA	after 24 hr.	12	33·00 (27·00–40·00)	64 (53·0– 77)	68·00 (62·00–74·00)
DTPA	on 3rd day	13	28·00 (23·00–33·00)	86 (63·0–117)	72·00 (67·00–82·00)
TTHA	on 3rd day	13	55·00 (48·00–63·00)	86 (68·0–109)	75·00 (67·00–84·00)
DTPA	on 5th, 7th, 9th day	18	11·00 (8·40–14·00)	70 (53·0– 91)	61·00 (53·00–70·00)
TTHA	on 5th, 7th, 9th day	18	29·00 (23·00–36·00)	74 (58·0– 95)	58·00 (52·00–65·00)

Table 2. INFLUENCE OF DTPA AND TTHA ON THE DISTRIBUTION OF YTTRIUM-91. THE ANIMALS WERE KILLED 3–6 DAYS AFTER THE INTRAPERITONEAL ADMINISTRATION OF THE CALCIUM CHELATES. THE DOSAGE, IF NOT OTHERWISE STATED, WAS 250 μMOLES/RAT

Treatment		No. of animals	Per cent of control Mean and fiducial limits ($P = 0.05$) Liver	Kidneys	Skeleton
DTPA	after 2 min.	12	36 (26–51)	23 (18– 30)	5·7 (4·9– 6·6)
TTHA	after 2 min.	12	42 (28–62)	25 (18– 33)	3·9 (3·6– 4·3)
100 DTPA	after 24 hr. (intravenous)	8	57 (45–71)	89 (54–149)	81·0 (74·0–87·0)
" TTHA	after 24 hr. (intravenous)	10	54 (44–65)	68 (43–107)	83·0 (77·0–89·0)
DTPA	on 3rd day	13	50 (39–66)	76 (46–126)	76·0 (70·0–83·0)
TTHA	on 3rd day	14	61 (47–79)	88 (54–144)	79·0 (74·0–85·0)

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containing the isotope would serve as a measure of the effect of environment. The results reported here are from preliminary experiments designed to assess certain aspects of the usefulness of this technique in studying calculi formation.

Rats were chosen as the experimental animal since it has been possible to form artificial calculi on zinc implants in this species readily[2,3]. It was deemed necessary to determine the urinary excretion of phosphorus-32 following massive doses of that isotope in order to establish whether or not the urinary level would subside rapidly enough to ensure that incorporation would become negligible between doses. Results from typical determinations of the excretion of urinary phosphorus-32 after a massive dose (40 μc./rat) are shown in Fig. 1. The results indicated that excretion of that isotope in the urine following administration decreased rapidly and was largely terminated after 8–10 days. The possibility of labelling single or a few laminations seemed feasible.

Thirty rats were therefore implanted with zinc pellets by techniques previously described[2] to induce calculi formation. The rats were fed 15 gm. of ground laboratory rat chow per rat per day. One group received 0·40 gm. of $CaHPO_4.2H_2O$ per rat per day as a supplement and other groups received 0·38 gm. of K_2HPO_4, or 0·086 gm. of tetraethyl silicate. An additional group served as controls.

The stones removed at autopsy (10 days after the second administration of phosphorus-32) were washed, dried and embedded in epoxy resin to serve as a mount. The mount was ground to expose the stones. Further careful grinding removed enough material to allow examination of a central transverse plane of the specimens. Microautoradiographs were prepared as previously described[1].

Fig. 2 shows an example of the mounted stones from the group receiving calcium phosphate supplement and Fig. 3 shows a microautoradiograph of the same stone. The results were encouraging. There was sufficient phosphorus-32 incorporated to provide adequate exposure for the nuclear-track plate in 4–5 days, in spite of the relatively short half-life of phosphorus-32. The apparent lack of incorporation into two separate concentric rings corresponding to each dosage period was due to the fact that these stones, as shown by diffuse, low-intensity mucicarmine staining, were not grossly laminated with alternate inorganic and mucoprotein layers. The lack of mucoprotein layers apparently allowed random exchange of phosphorus to occur throughout the stone. Stones which were removed from other rats of the other groups and which contained enough deposit to yield a satisfactory microautoradiograph were similar to that shown.

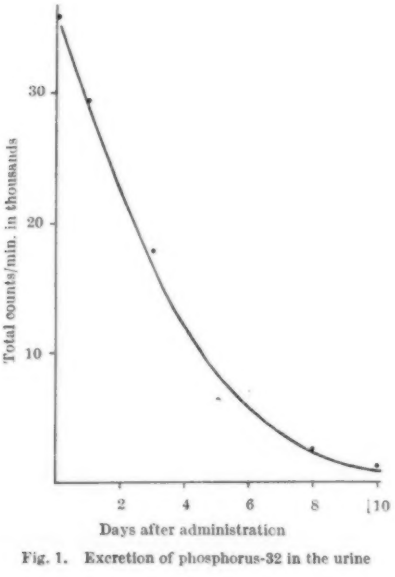

Fig. 1. Excretion of phosphorus-32 in the urine

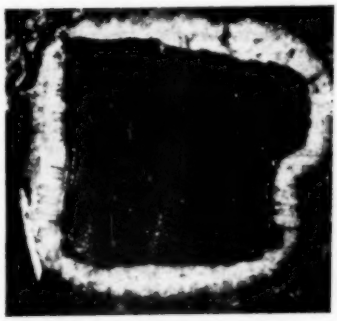

Fig. 2. Typical mounted rat calculus showing the zinc implant in the centre with the concretion surrounding the implant

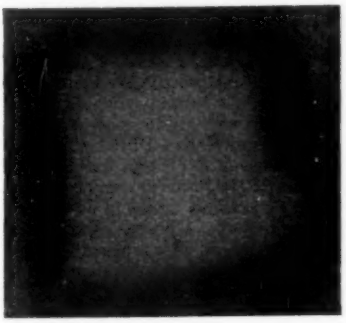

Fig. 3. Microautoradiograph of the stone shown in Fig. 2

Incorporation of phosphorus-32 was more than adequate for microautoradiography. It was somewhat lower in the rats receiving a tetraethyl silicate supplement than in the others. With silicate supplement, the stones were probably of the high-silicon but low-phosphorus type[3], and hence less incorporation would be expected. None of the stones was grossly laminated, and therefore showed only slight separation into distinct rings labelled with phosphorus-32.

If present experimental work on formation of siliceous calculi on zinc implants in the bovine species provides an adequate means of producing such calculi, then it is anticipated that the phosphorus-32 labelling technique will be very useful. Most siliceous bovine stones are strikingly laminar[1]; this is not the case with stones from rats, and bovine stones do contain enough phosphorus to ensure that sufficient incorporation of phosphorus-32 would be expected with little inter-laminar exchange. These preliminary results suggest that the method offers promise in the study of the rate of formation of bovine calculi under experimental conditions.

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HÆMATOLOGY

Haptoglobin Patterns in Cord Blood Serums

ALTHOUGH haptoglobin is almost always present in normal adult serums, it is demonstrable in only about 10 per cent of cord blood serums by the technique of starch-gel electrophoresis[1]. The factors accounting for this large percentage of ahaptoglobinæmic new-borns are obscure. The present communication is based on a survey of cord-blood serums undertaken in an attempt to evaluate some of the factors accounting for the high frequency of ahaptoglobinæmia in the new-born. At the same time, work was carried out to substantiate that the new-born baby with detectable haptoglobin produced it himself.

Two hundred and twelve cord blood serums were subjected to the horizontal starch-gel electrophoresis technique of Smithies[2], using Poulik's discontinuous buffer system[3]. Before electrophoresis, sufficient hæmoglobin was added to each serum to saturate completely any haptoglobin present. One half of each resultant gel pattern was stained with amido black 10*B* to detect protein bands, and the other half with a dilute benzidine stain to locate the haptoglobin–hæmoglobin complexes[4].

Of the 212 cord blood serums examined, 27 (13 per cent) had detectable haptoglobin. As had been noted[5], the patterns, though characteristic, were frequently faint or not detectable with the amido black, and clearly definable only with the more sensitive benzidine stain. Haptoglobin typing of these new-born babies' parents revealed no exceptions to the genetic hypothesis of Smithies and Ford-Walker[6]. No unusual haptoglobin types were encountered.

Comparison of hæmoglobin, hæmatocrit and serum bilirubin-levels between the ahaptoglobinæmic new-borns and those with detectable haptoglobin revealed no evidence to suggest that *in vivo* hæmolysis caused ahaptoglobinæmia (Table 1).

Of fourteen cord blood serums examined from new-born babies with birth-weights less than 2,500 gm., three showed haptoglobin. The smallest tested, with an estimated gestational age of 28 wk. and a weight at birth of 1,106 gm., had haptoglobin of the 2–2 type. The mother had haptoglobin type 2–2 as well.

Table 1. COMPARISON OF NEW-BORN BABIES WITH AND WITHOUT HAPTOGLOBIN

Determinations*	Ahaptoglobinæmic new-born babies			New-born babies with haptoglobin		
	No. tested	Mean	Range	No. tested	Mean	Range
Hæmoglobin, gm. per cent	182	17·3	12·0–23·2	27	16·9	13·6–21·2
Hæmatocrit, per cent	180	58	40–78	26	59	45–80
Serum bilirubin, mgm. per cent	182	6·4	1·0 –18·0	26	5·7	2·0–14·0

* Performed at 2 days of age.

A higher number (13 of 27) of new-born babies with *Hp* 1–1 was found than would be expected in a randomly selected, predominantly Caucasian population such as this series represented. This was probably due to the greater technical ease of detecting the single band *Hp* 1–1 type when the total serum haptoglobin concentration is low.

In 12 of the 27 instances of cord blood serums with detectable haptoglobin, the mother's type differed from that of her offspring. This finding is indicative of the production of haptoglobin *in utero* by these twelve new-born babies. Of these twelve heterospecific baby–mother haptoglobin combinations, nine new-born babies showed *Hp* 1–1. For these nine it might be argued that the smaller molecular weight haptoglobin 1 species crossed the placenta from their *Hp* 1–2 mothers, to give *Hp* 1–1 patterns in the new-born serum. However, in the remaining three heterospecific baby–mother combinations, the new-born was *Hp* 1–2. The mothers of two of these had *Hp* 2–2 patterns, so their offspring must have produced the *Hp* 1 species of molecule *in utero*. The mother of the third new-born had *Hp* 1–1, giving evidence that her *Hp* 1–2 offspring must have produced the *Hp* 2 species of molecules under its own genetic control. This evidence for the fœtal production of haptoglobin corroborates the findings of Galatius-Jensen, who reported four mother new-born haptoglobin combinations with differing types in mother and baby[5].

We thank Dr. Stewart H. Clifford, project director at the Boston Lying-in Hospital, for making the hæmoglobin, hæmatocrit and bilirubin values available to us.

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Serum Factor *Gm*^{a} among the Iranians

OF all the genetically determined serum constituents which have been called *Gm* factors, the factor *Gm*^{a}, discovered by Grubb[1], is the most frequently studied from the point of view of its distribution among different populations. Hitherto, the proportion of *Gm*(*a*+) subjects found among persons of the white race has varied between 67 and 39 per cent (Table 1).

We have examined 296 Iranian subjects, coming from different regions, whose blood samples were taken at the Transfusion Centre of the Iranian Army, at the time of their medical examination for military service. These subjects were twenty years old and apparently in good health.

Table 1. The Serum Factor Gm^a among the Various Populations of the White Race

Population	No. tested	$Gm(a+)$ (per cent)	$Gm(a-)$ (per cent)	Ref.
Lapland	110	67·3	32·7	1
Finland	477	65	35	4
England	133	61	39	5
Norway	320	60·6	39·4	6
Sweden	360	59·7	40·3	7
Australia	100	58	42	Kirk, 1948
Denmark	1,084	55·6	44·4	8
France	300	57·3	42·7	3
France	1,000	51·7	48·3	9
France	300	54·3	45·7	10
Italy (south)	125	40·2	59·8	5
Greece	308	39	61	9
Poland	167	39·5	60·5	12

The sera were separated from the clots at the Transfusion Centre, put in sterile bottles, sealed and sent by air to the laboratory for study.

The determination of the factor Gm^a was carried out by the tube technique with the same anti-*Rh* serum as that used in our previous work[2,3], and with the same serum from patients suffering from rheumatoid arthritis. These reagents, as well as the erythrocyte samples coming from the same donors, had thus been previously selected and controlled.

Out of the 296 sera examined, we found 163 $Gm(a+)$ and 133 $Gm(a-)$, that is, 55·4 per cent $Gm(a+)$ and 44·6 per cent $Gm(a-)$ respectively.

As the subjects examined came from different parts of Persia, the distribution was calculated separately for each of the 13 groups which could be defined.

The results for seven of them are presented in Table 2. The number of individuals coming from other regions (Mazadran, Kermanchah, Pharse, Kurdestan, Gorgan, Kerman and Yazd) was too small to allow the calculation of the frequency to have any significance.

Table 2. Distribution of the Gm^a Factor in the Different Provinces of Iran

	No. tested	Number $Gm(a+)$	$Gm(a+)$ (per cent)
Azerbaijan	48	26	54·1
Gilan	45	25	55·5
Tehran	57	31	54·3
Isfahan	38	21	55·2
Kashan (QOM)	27	16	59·2
Hamadan	19	9	47·3
Khurasan	19	12	63·1

Table 3. Relation between the Gm^a Factor and the Blood Groups

	No.	Per cent	$Gm(a+)$		$Gm(a-)$	
			No.	Per cent	No.	Per cent
A	89	30·1	44	27·0	45	33·8
B	71	24·0	45	27·6	26	19·6
O	115	38·8	63	38·6	52	39·1
AB	21	7·1	11	6·8	10	7·5
Total	296		163	55·4	133	44·6

So far as the relations between the factor Gm^a and the antigens of the blood groups *A*, *B*, *O* are concerned, the distribution gave us a value of χ^2, which was not significant.

A quantitative determination of the γ-globulins was carried out on each of the samples of serum and, as in previous work, no notable differences were observed between the average percentages of γ-globulins of $Gm(a+)$ and $Gm(a-)$ subjects.

Thus, the distribution of Gm^a factors among the Iranians is nearly the same as that previously found for persons of the white race.

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HISTOCHEMISTRY

Histochemical and Homogenization Studies of Mitochondrial α-Glycerophosphate Dehydrogenase in the Nervous System

An insoluble brain enzyme capable of oxidizing α-glycerophosphate[1] has been shown to be part of a purely mitochondrial system which resembles succinate dehydrogenase in that pyridine nucleotides do not participate in electron transfer[2]. Histochemical localization of this activity in mammalian nervous tissue was made difficult by the fact that tetrazolium salts are ineffective electron-acceptors in preparations which oxidize α-glycerophosphate. Maximal reaction-rates, however, were obtained by using 2-methyl-1,4-naphthoquinone (menadione, K_3) as intermediate acceptor. Histochemically, this effect was first demonstrated by Wattenberg in liver slices[3]. We found that menadione could be substituted by the sodium bisulphite derivative, but the diphosphoric acid ester ('Synkavite'), vitamin K_1, and 1,4-benzoquinone were ineffective.

In the presence of reduced menadione (K_3H_2), tetrazolium salts are reduced to formazan pigment. This reaction, which is non-enzymic[4], renders histochemically visible the reduction of K_3 by its reductase. Formazan formation by K_3H_2 occurred above *p*H 6·1 and at neutral and slightly alkaline *p*H; this reaction could compete successfully with both auto-oxidation of K_3 and the activity of K_3H_2-oxidase (compare ref. 5). At *p*H 7·4 we found the rate of tetrazolium reduction by rat brain mitochondria in the presence of K_3 to be about 50 per cent higher with α-glycerophosphate than with succinate as substrate. This result compares favourably with measurements of respiration-rate in the presence of oxygen[5].

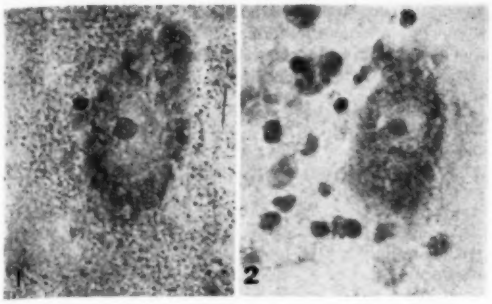

Fig. 1. Rat spinal cord. α-Glycerophosphate–menadione reductase activity in oligodendrocytes. The inactive motor ganglionic cell is counter-stained with thionine. (× 560)
Fig. 2. As Fig. 1, but activity inhibited by addition of phenyl-indane-dione. (× 560)

Histochemical reactions were carried out on 2–8μ fresh frozen sections of rat tissue which were incubated for 15 min. at 22° in media containing substrate (10^{-2} *M*), 3-(4·5-dimethylthiazolyl-2)-2·5-diphenyl tetrazolium bromide (MTT) (10^{-3} *M*), cobalt chloride (10^{-3} *M*), *tris*(hydroxymethyl)aminomethane buffer *p*H 7·4 (0·1 *M*), sucrose (0·25 *M*), polyvinylpyrrolidone (1 per cent). High activity with α-glycerophosphate as substrate was observed in neuroglia (Fig. 1) in cells of choroid plexus, in ependyma and in vascular structures. Moderately strong activity was observed in ganglionic cells in certain areas (for example, spinal ganglia); motor neurones were inactive.

Quantitative studies were carried out on rat brain homogenate using the histochemical systems and the results are shown in Table 1. The specific activity of washed brain mitochondria (prepared according to ref. 7) was compared with the activity of the remainder of the cytoplasmic fraction containing soluble flavoproteins.

Table 1. Tetrazolium Reduction by Rat Brain Fraction in Presence or Absence of Menadione*

Substrate	Addition	Mitochondria *a*†	Mitochondria *b*†	Cytoplasmic fraction
α-Glycerophosphate (10^{-2} *M*)	none	0·15	0·06	—
	K_3	0·95	0·91	—
Succinate (10^{-2} *M*)	none	0·22	0·26	—
	K_3	0·47	0·47	—
DPNH (10^{-3} *M*)	none	0·42	0·40	0·20
	K_3	1·16	1·50	1·10
TPNH (10^{-3} *M*)	none	0·22	0·22	0·25
" "	K_3	1·24	1·10	1·15

*Activity expressed as μgm. formazan/mgm. protein/min.
†*a*: Freshly prepared; *b*, frozen-thawed twice.

All the enzyme reactions tested were sensitive to both dicoumarol (10^{-4} *M*) and *p*-chloromercuribenzoate (10^{-5} *M*), thus resembling the reactions of a purified brain diaphorase[8]. Selective reduction of the K_3-dependent increase in mitochondrial activity was produced by 2-phenyl-indane-1 : 3-dione (PID) (5×10^{-4} *M*), both *in vitro* and histochemically (Fig. 2). It is likely that PID exerts a similar action on various mitochondrial systems in other organs, since an antagonistic effect of K_3 and PID on uptake of iodine-131 by rat thyroid slices has been observed by Williams and Doniach (unpublished results). Wosilait[9] has demonstrated different inhibitory activity of dicoumarol and PID on vitamin K_1-reductase from dog and rat liver.

Amobarbital can act as inhibitor of both reduced diphosphopyridine nucleotide (DPNH)–coenzyme *Q* reductase[10,11] and purified brain diaphorase[8]. In our experiments its sole effect (at 5×10^{-4} *M* concentration) was to abolish the K_3-induced increase in mitochondrial tetrazolium reduction with succinate and α-glycerophosphate as substrates. On the other hand, mitochondrial activity with pyridine nucleotide-linked systems was not affected. Antimycin *A* partially inhibited direct tetrazolium reduction with α-glycerophosphate and succinate as substrates, but did not impair formazan production via K_3 as the intermediate electron carrier. With DPNH and reduced triphosphopyridine nucleotide (TPNH) as substrates, no effect of antimycin *A* on the rate of formazan formation could be demonstrated in the presence or absence of K_3.

The extensive glial activity of mitochondrial α-glycerophosphate dehydrogenase is of interest in view of the important part played by mitochondrial glycolysis in brain metabolism[12]. The histochemical reaction involving K_3 and α-glycerophosphate probably uses a pathway different from that leading to direct enzymic tetrazolium reduction. In accordance with Wosilait and Nason[12], the present enzyme should be termed α-glycerophosphate-menadione reductase.

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HISTOLOGY

Ethylene Glycol Monomethyl Ether, a New Fixative for Histological Work

While studying the guinea pig mast cell[1], we have discovered a new chemical family of histological fixatives, namely, the glycol ethers. These alcohol derivatives, possessing both an ether and an alcohol group, are miscible with most liquids and are excellent solvents for a variety of substances completely insoluble in water. Marketed under the trade names of 'Cellosolves' and 'Carbitals', they have found wide use as solvents for cellulose esters in the lacquer industry. Conversely, they do coagulate and precipitate proteins as well as mucopolysaccharides.

Our initial observations were made on the guinea pig mesentery, with special reference to the labile water-soluble mast cell. Excised specimens were immediately placed in the test solutions (100 per cent

concentration, ice cold) and remained there for 18 hr. Afterwards, staining of this naturally thin membrane was achieved with methylene blue (0·25 per cent aqueous, 3 min.), dehydration in absolute ethyl alcohol (2 min., twice), clearing in xylene (5 min., twice), and whole mounts made in a synthetic resin medium (*HSR*).

Ethylene glycol monomethyl ether acetate proved to be the best coagulant fixative of seventeen members of the glycol ether family which were studied. It not only regularly gave solid blue-black mast cells, but also produced a highly desirable intensification of the general staining. The other mesenteric cells showed fine nuclear detail, and collagen fibres were distinct. Significantly, the parent substance, 'Cellosolve' (ethylene glycol mono-ethyl ether), was also an excellent fixative of the mast cell and its use led to almost selective staining of the mast cell. The mesentery and its other cells stood as a pale almost non-staining background for the vivid blue-black mast cells. Here the distinct basophilic granules were in evidence and at times the nuclear space was apparent as a non-staining vacuole. The polymorphism of the mast cell was particularly striking. In the control specimens degranulation was not seen. When such mast cell discharge was induced pharmacodynamically, the fixative faithfully captured the free granules.

The excellence of these fixatives for the guinea pig mesentery led us to explore the general usefulness of the glycol ethers in fixation of other mammalian tissue, using standard paraffin embedding and staining procedures. Using normal as well as diseased human skin, as a test object, it was found that ethylene glycol monomethyl ether produced superior fixation of the cellular components even at room temperature. Nuclear detail, colour brightness and contrast, and glycogen fixation were particularly attractive attributes of the slides prepared from 100 different biopsies. However, in some specimens polarization or streaming of the cytoplasm was seen. A variety of stains were used with consistently fine results and it was found that washing out of the fixative was not necessary.

Despite the rather awesome list of available fixatives[2], Baker has pointed out that the number of substances really useful in chemical fixation is still very small[3]. Accordingly, ethylene glycol monomethyl ether and certain of its congeners are recommended as new non-aqueous fixatives deserving of further trial in histological and histochemical work. They may prove particularly rewarding in fixed tissue enzyme assays of water-soluble components. In our laboratory they have been of continuing value in the fixation and staining of mast cells.

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BIOLOGY

Adaptive Value of Regenerative Ability

The view that the distribution of regenerative ability among and within animals is not correlated with the incidence of loss or damage to their organs, and so with survival value, rests very heavily on the work of Morgan[1] on the hermit crab, *Eupagurus longicarpus*. He concluded that the regenerative ability of its various appendages was not correlated with their liability to loss: in particular, the last two pereiopods (thoracic walking legs) and the abdominal appendages, all of which are protected inside the host shell and are rarely lost, regenerate well. He also found that limbs regenerate not only at their autotomy plane, classically regarded as an adaptation to facilitate both escape and regeneration, but also when amputated proximally or distally to the plane. Recent work[2,3] has tended to confirm in some detail the neo-Darwinian view that regenerative ability is correlated with its utility, as measured by the incidence of natural losses. However, since considerable emphasis has again[4] been placed on Morgan's conclusions, it seems worth re-examining his results in the light of a survey made a few years ago (August 1955) on *E. bernhardus*.

Eighty-two individuals, 42♂ and 40♀, were collected at Seaton, south-east Cornwall. They were removed from the shell after fixation in alcohol and examined under a low-power binocular microscope for fresh and long-standing (natural) losses of eyes, first and second antennæ, pereiopods and abdominal appendages. Long-standing losses include those in the course of regeneration. Recent losses are due mainly to irritation by the fixative. Of the abdominal appendages, the pair of uropods and the left-side members of segments, 3, 4 and 5 are normally present in both sexes. In addition, the left appendage of segment two is normally present in the female, all others being absent in both sexes.

The flagellum of the second antenna fractures readily at any point and the distal end was found missing in most individuals. In addition, eleven showed a recent loss. Losses to the other appendages were:

Table 1

Appendages	♂		♀	
	Fresh	Long-standing	Fresh	Long-standing
Eyes	0	2	0	3
Antennules	0	1	0	1
Pereiopods				
1 (chelæ)	7	5	7	2
2	0	0	3	0
3	0	1 (tip)	2	0
4	0	0	0	0
5	0	0	0	0
Abdominal				
2	normally	absent	0	0
3	3	0	0	0
4	1	2	0	0
5	0	1	0	0
6 (uropods)	0	0	0	0

The incidence of loss is seen to be related to the degree of exposure of the appendages, but with one outstanding exception, the abdominal appendages of the male. Morgan also found some of these appendages missing, but dismissed the matter lightly . .

"it is possible that the (abdominal) appendages may be occasionally lost". His percentage of losses (4 in 100 crabs) was somewhat less than half that among the pereiopods (29 in 288 crabs), both percentages being similar to those of the present analysis. It was therefore unwarranted to conclude that the ability of the abdominal appendages to regenerate has no useful biological significance.

Morgan claimed that these appendages regenerate virtually as well as the anterior pereiopods, but in fact he had only 10/47 (21 per cent) successful regenerations compared with 10/12 (92 per cent) for the latter. Similar quantitative differences are evident between his records for regeneration at the autotomy plane and those for regeneration elsewhere in a limb. These last results are in essential agreement with more recent results for the thoracic limb of *Asellus*[5], in which the distribution of regenerative ability seems adaptive in every detail. Morgan tended to minimize quantitative differences in ability and to magnify differences in susceptibility to loss.

If he had measured all as carefully as his data permitted, he could have brought out more clearly the unique properties of the uropods and the last two pairs of pereiopods. These properties are confirmed by the present results and by themselves present a far more serious challenge to the 'adaptation' theory. These appendages all regenerate very well, yet are rarely found missing or regenerating. The right-hand pereiopods 4 and 5 appear to be usually shorter and paler than those on the left and the short right uropod is sometimes paler and possibly thinner-shelled than its partner, but these may be normal ontogenetic aspects of the general asymmetry of the posterior part of the body and there was no completely convincing case, in the present results, of natural losses in the process of regeneration.

In explanation of the behaviour of these limbs, it is important to appreciate their indispensable role in movements in and out of the gastropod shell and in anchoring the hermit inside it. The loss of one limb renders its partner also useless, and probably all three pairs are individually indispensable. This would be an adequate adaptive basis for both their high regenerative ability and their lack of autotomy mechanisms. Incidence of loss is related directly to exposure, but also inversely to the indispensability of the appendage. This may explain also why the female, unlike the male, does not lose her other abdominal appendages, which are used to carry the 'berry' after oviposition. A parallel case is the fifth thoracic limb of the mature male *Asellus*[6], both members of the pair being essential to hold the female during the (considerable) period before coition. The limbs autotomize readily in females and immature males. Like the *Eupagurus* limbs in question, the limb of the mature male regenerates well if experimentally amputated.

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Simultaneous Eradication of Three Ectoparasitic Species from a Colony of Laboratory Mice

The mange mites and blood-sucking louse commonly infesting laboratory mice (to the almost certain detriment of survival and fecundity) can be entirely eradicated by modern topical and systemic pesticides. The present communication gives details of a proved, if time-consuming, procedure whereby a one-room colony of four hundred cages was cleared of three of the following ectoparasites (Fig. 1):

Mange mites. (i) *Myocoptes musculinus*[1], off-white; massive brown claspers on third and fourth pairs of legs; concentrates on the body, especially neck and rump; may thickly encrust the skin; clusters of pink eggs on each hair often discolour the coats of albinos. Eradicated.

(ii) *Myobia musculi*[1], all-white: claspers on first pair of legs; concentrates on the head, especially at the base of whiskers and eye-lashes; sometimes causes weeping sores; white eggs, one at the base of any hair. Eradicated.

(iii) *Psorergates simplex*[2], white; no claspers; all stages found together within pustules under the skin, especially facial skin; severe manifestation[3] rare. Virtually eradicated.

Blood-sucking louse. (iv) *Polyplax serrata*, 1 mm. long; highly mobile; silver eggs attached half-way along the hairs. Eradicated.

Prospects of disinfestation. Residual infestations remained after sulphur dust[4], lindane[5,6], tetmosol[6] and dibutylphthalate[3] had been used as whole-body treatments, and none seemed capable of disinfesting an entire colony. Only tetmosol caused no distress to the mice; it was also observed to kill *Polyplax* instantly.

However, at least four pesticides untried by me have some prospect of eradicating all the above mouse pests. The simplest to use would be the systemic

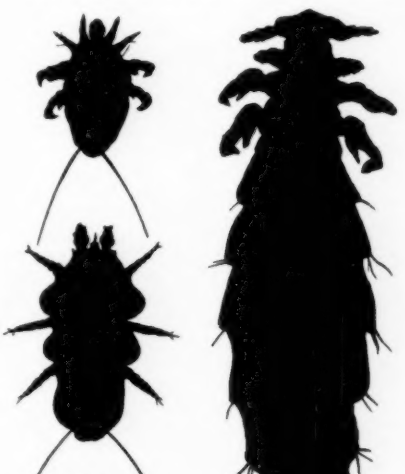

Fig. 1. Representations to scale of the four ectoparasitic species on laboratory mice. The largest is the louse *Polyplax*; the others, from top to bottom, are the mites *Myocoptes* ♀ (the abdomen of the male scarcely protrudes beyond the last legs), *Myobia* and *Psorergates*

pesticides tedion[7,8] and trolene[9], both widely applied to farm animals, if suitable doses for their addition to the food or water can be found for mice. Otherwise, the topical pesticides chlordane or malathion[10] could be tried. A single application of chlordane to fresh bedding is known to eradicate *Polyplax* from mice[4], and the murine mites might be equally susceptible, since those on hamsters were considerably reduced in numbers by the remote action of chlordane painted on to the trays of their cages[11]. Similarly for malathion: a single dip killed all *Myocoptes*[12]; routine treatment of bedding cured a colony of myocoptic and myobial manges where mice were frequently introduced from outside sources[5]; and it might prove effective against *Polyplax*, having been used successfully against lice on birds[13].

The present method uses an aqueous dip of tetmosol and DMC. DMC had already been used in 50 per cent alcoholic solution, first to free a small colony from *Myocoptes*[14], and afterwards a much larger colony from *Myocoptes*, *Myobia* and *Polyplax* (Carter, T. C., and Lyon, M. F., personal communication). In these cases, each mouse was bathed three times at five-day intervals, and very great care was needed to ensure survival. For this reason, after the first room of the larger colony had been disinfested, mice from the remaining rooms were treated at birth by removing the mites with forceps, swabbing the mice once only with the DMC solution, and then fostering them immediately on to already disinfested mothers (Carter, T. C., and Lyon, M. F., personal communication). But disinfestation can be more casual if DMC and tetmosol are presented together in aqueous emulsion, using the tetmosol both to augment the miticidal action of DMC and to be the effective insecticide in the absence of alcohol.

A dip was made by mixing: 2 gm. DMC dissolved in 3 gm. ethanol; 67 gm. tetmosol, and 1 litre warm tap-water. The gift of the DMC, or di-(*p*-chlorophenyl)methylcarbinol, by Sherwin Williams Inc., Cleveland, Ohio, is gratefully acknowledged; the tetmosol, a 25 per cent solution of tetraethylthiuram monosulphide, was supplied by I.C.I. (Pharmaceuticals) Ltd., Wilmslow, Cheshire. The dip was maintained at 30°–37° C. in a water-bath no hotter than 40° C., otherwise mice falling into it could be killed. Mice were given two dippings 1 hr. apart and two further dippings 1–3 weeks later. The first and third dippings were prevented by air pockets from killing every parasite but made the skin thoroughly receptive to the dip on the next occasions; the third and fourth dippings mopped up rare survivors. On each occasion the mice swam for half a minute and were submerged about six times, were drained for three-quarters of a minute while the next mouse was swimming, and then returned to their cages—to their old cages after the first and third dippings, and to clean cages after the second and fourth. A few severely chilled mice had to be specially revived in warm air.

Disinfestation was started when no new litters were immediately expected and the mice could be treated as adults or well-grown weanlings. All mice had the first two dippings within 5 weeks, and many stocks were by then completely disinfested. About a quarter of the mice were required for breeding before their final dippings could be given, and complete disinfestation of these stocks was deferred up to six months and applied to later generations. During this time the disinfested stocks were isolated so far as possible on the upper shelves, and hands were scrubbed before handling them. But no lice and very few mites survived the ancestral second dipping, and recontamination was not observed. The full treatment averages 3 min. per mouse, but much time might be saved by dipping only once in a more concentrated and penetrating emulsion to which a harmless wetting agent such as 1 per cent sodium alkylaryl sulphonate[3] had been added, and by treating several mice together in baskets constructed for the purpose.

Altogether, 1,700 mice were treated without loss. About one thousand of these became parents and were minutely inspected under a stereoscopic microscope at autopsy. In subsequent generations a similar number, representing 10 per cent of sires, have been inspected on the face and back. No *Myocoptes*, *Myobia* or *Polyplax* have been seen in the 2½–3 yr. since treatments were completed, nor have *Psorergates* pustules been recognized in the past half year.

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A Natural Hybrid of *Tilapia nigra* and *Tilapia leucosticta* from Lake Naivasha, Kenya Colony

Cichlid fish of the genus *Tilapia* are of considerable importance in both natural fisheries and pond culture in many tropical and subtropical climates, particularly throughout Africa. Certain species have proved to be adaptable to varied environmental conditions, and consequently there has been a considerable artificial dispersion of species beyond their natural geographical range, as far afield as Malaya and Pacific islands, and including high-altitude waters in several parts of Africa. This transportation has brought into contact species which were hitherto geographically isolated, and due, at least in part, to this, there is an increasing number of records of hybridization. Lowe[1] first directed attention to possible hybridization between *T. nilotica* (Linné) and *T. nigra* (Günther), and between *T. nilotica* and *T. esculenta* Graham in Uganda. Yashouv and Chervinsky[2] have crossed *T. nilotica* and *T. galilea* (Artédi) in Israel, and Bard[3] reports a hybrid between *T. nilotica* and *T. machrochir* Boulenger in culture ponds in the Cameroons. More recently, Hickling[4,5] crossed a Malayan strain of *T. mossambica* Peters with another of the *T. mossambica* group from Zanzibar, and Mortimer[6] has hybridized *T. mossambica* and *T. andersoni* (Castelnau) in culture ponds in Northern Rhodesia.

Excluding Lowe's[1] unconfirmed observations, none of these hybrids has occurred in 'natural' waters. The crosses recorded by Hickling[5] and Mortimer[6] were

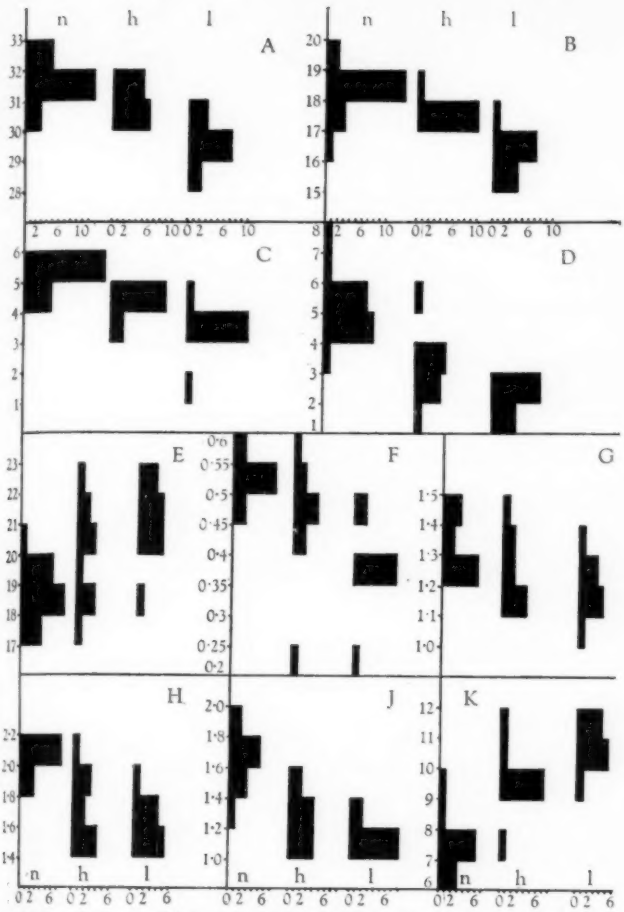

Fig. 1. *Abscissæ:* all figures from left to right, (*n*) *T. nigra*, (*h*) hybrids, (*l*) *T. leucosticta*, as numbers of fish.

Ordinates: (*A*) Lateral line scales. (*B*) Dorsal fin spine number. (*C*) Anal fin spine number. (*D*) Extent of pectoral fins: (1 < vent, 2 < papilla, 3 < anal spine I, 4 < spine II, 5 < spine III, 6 < spine IV, 7 < spine V, 8 < spine VI). (*E*) Gill raker count. (*F*) Ratio of length/width of tooth pad on lower pharyngeal bone. (*G*) Ratio of length/depth of premaxillary. (*H*) Ratio of length/depth of dentary. (*J*) Ratio of length/width of dentary. (*K*) Ratio or standard length/length of dentary bone

the results of deliberate attempts to produce improved strains for fish culture work.

In all the instances mentioned, the species belong to the 'maternal brooder' group of *Tilapia*, in which the female normally broods the eggs and young in her mouth.

In East Africa, *T. zillii* (Gervais), a macrophyte feeder, has been more widely dispersed than most other species, in the hope that it will clear weed-choked waters; and Whitehead[7] has described a cross between the female mouth-brooding species *T. nigra* and the 'substratum-spawning' *T. zillii* in Lake Naivasha and also in a flooded rice paddy field at the Tebere Rice Irrigation Scheme, Kenya. Lake Naivasha is a shallow basin of some eighty square miles, which originally contained only one species, *Aplocheilichthys antinorii* (Boulenger), endemic to Lake Naivasha and southern Ethiopia. During the past forty years, various other species have been introduced: *T. nigra* was stocked to provide forage for the introduced black bass (*Micropterus salmoides* (Le Seur)). Rainbow trout (*Salmo gairdnerii* (Richardson) have also gained access from the affluent rivers and *Lebistes* and *Gambusia* were introduced for mosquito control. Most recently, *T. zillii* was stocked from ponds in Kisumu, Kenya.

In postulating hybridization between *T. nigra* and *T. zillii*, Whitehead (unpublished work) noted certain similarities between a proportion of his hybrids and *T. leucosticta* Trewavas, although the agreement was not complete. On the basis of Whitehead's[7] communication and some of his unpublished results, Trewavas and Greenwood[8] suggested that the hybrids were in fact *T. leucosticta*, another mouth-brooding species, which had gained access to Lake Naivasha by accidental inclusion in the *T. zillii* stocking. In addition, they did not exclude the possibility of some hybridization having occurred between *T. nigra* and *T. leucosticta*.

The occurrence of both pure *T. leucosticta* and hybrids between *T. leucosticta* and *T. nigra* has been confirmed by more recent work, on Lake Naivasha.

During a routine analysis of some five thousand fish, numerous specimens were collected, the taxonomic characters of which agree in detail with the description of *T. leucosticta* given by Lowe[9]. In addition, however, eleven specimens, of 28–31 cm. total length, were recognizably distinct from both *T. leucosticta* and *T. nigra*, and showed a variable complex of intermediate characters. The field characteristics on which they were originally distinguished were depth of body, profile of head, shape and size of mouth, count of anal fin spines and coloration. Further examination of the meristic and morphometric characters of these specimens confirmed their intermediacy, and a summary of the principal characters is given in Fig. 1.

Confirmation of the hybrid identity has not yet been attempted by artificially crossing *T. nigra* and *T. leucosticta*, but there can be little doubt that hybridization has occurred, and that Whitehead's[7] original description was based on a mixture of pure *T. leucosticta* and *T. nigra* × *T. leucosticta* hybrids. The fact that all these hybrids were mature males and the low proportion in the total population may invite speculation regarding their viability. This cannot be established until a very much wider size-range has been examined, since sexual dimorphism occurs in the growth of both *T. nigra* and *T. leucosticta*, the males being somewhat larger.

This investigation was undertaken as a necessary preliminary to a study of the fishery potential of Lake Naivasha. It is now possible to identify *T. nigra*, *T. leucosticta*, *T. zillii* and *T. leucosticta* × *T. nigra* hybrids in a population formerly considered to be pure *T. nigra* and *T. zillii*. However, the exact taxonomic identity of a large proportion of the Naivasha *Tilapia*

still remains to be determined, owing to the very wide and variable range of colour, meristic and morphometric characters which has been encountered.

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Further Observations on a Filamentous Saprophyte from Wookey Hole Caves

Work now completed on this unusual organism, found growing in darkness in rather stagnant water of Wookey Hole Caves, Somerset, has yielded some information additional to that reported earlier[1]; but the identity of the saprophyte remains unknown.

The organism, although slow-growing, may be successfully cultured in the light or in darkness on a range of solid and of liquid media, and can readily utilize glucose, sucrose and mannose. A notable feature is its great plasticity of form, which may vary, dependent on the cultural conditions, from septate, branching filaments to chains of rounded cells which almost separate from one another. X-ray diagrams of the cell wall show some similarity to those of yeasts. Usually there are several very small, obscure nuclei per cell, and photosynthetic pigments are lacking. On certain media the organism produces large amounts of oil, especially in old cells; thick-walled chlamydospore-like bodies, which give rise to fine filaments on germination, are formed in some cultures. On media low in glucose, anastomoses are frequent; however, no sexual organs have been observed, despite the wide range of cultural conditions and treatments investigated.

A full, illustrated account of this saprophyte, which seems to show more affinity to the fungi than to the algae, will be given elsewhere[2].

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Axenic Seedlings of *Drosera*

Carnivory in higher plants fascinated Darwin[1] and later students of evolution, yet a century-long debate[2] on the extent of *Drosera's* dependence on, and specialization for, insect prey remains inconclusive. A technique for obtaining axenic *D. intermedia* (a sundew) is reported here—a step forward in analysing its biochemical capabilities.

Büsgen[3] preferred seedlings to winter buds as more uniform experimental subjects. Vegetative plantlets are better, as they circumvent genetic variation. However, for purposes of aseptic cultivation, seeds—easier to handle and protected by their hard coat—are best, assuming that vegetative propagation follows. Seeds were collected in September (between ripening and shedding) at Bethel, New York, and stored dry at room temperature for 8 months. For sterilization, seeds were immersed in an aqueous solution of 'Clorox' (5 per cent) plus 'Aerosol' *OT* (0·02 per cent) for 10 min. with constant shaking, then washed three times in sterile distilled water and inoculated in batches of 10–12 from the water to agar slants (composition in Table 1).

Table 1. Composition of Slant Media

Compound	mgm./100 ml. final medium
NH_4NO_3	12·0
KH_2PO_4	6·8
$MgSO_4.7H_2O$	12·0
$Fe(NH_4)_2(SO_4)_2.6H_2O$	0·56
$CaCO_3$ (dissolved in HCl)	2·0
Sucrose	2,000·0
Trypticase (Baltimore Biological Lab.)	50·0
'Complete Supplement No. 10'*	0·5 ml./100
Agar	1,000·0
*p*H 6·0	

* An aqueous solution of *B* vitamins, amino-acids, hydrolysed nucleic acids, and an acid hydrolysate of casein (Hycase, Sheffield) quite similar to 'complete supplement No. 9' given in ref. 4.

For uniformity, seeds for each experiment were sterilized together. Viability of unsterilized seeds was not determined. Of 3,200 seeds on slants stored 3 months at room temperature in the dark, only two germinated. Exposure to indole-3-acetic acid, gibberellic acid, dilute sulphuric acid, high temperature (37° C.), or freezing followed by either high or room temperature, did not enhance germination.

However, several slants left on a west-facing window-sill in September showed 6 per cent germination after one month, suggesting physical germination stimuli. *D. intermedia* in southern New York State normally germinates in March and April on the moist surface of acid bogs. Temperature- and light-alternation, reported stimulatory to germination in various other plants[5], were tried. Freshly inoculated slants were subjected to a cycle of 12 hr. at 15° C. and 12 hr. at 38° C., and either illuminated by two 40-W. 'cool-white' fluorescent lamps or kept in the dark when desired, by wrapping the tubes. Germination was observed after 5 weeks of treatment. As shown in Table 2, seeds subjected to a temperature-cycle plus light showed significant germination, but not when exposed to constant temperature or placed in the dark, at any temperature. No dark period is necessary.

Table 2. Effect of Light and Temperature Alternation on Germination of *D. intermedia*

	Percentage germination*		
	Constant		Alternate Light and dark
	Light	Dark	
15° C. constant	0	0	0
38° C. constant	2	0	0
15°–38° C. alternation	44	2	31†

* Each sample contained about 100 seeds.
† In the temperature–light alternation, the illuminated phase was at 38° C., the dark at 15° C. The reciprocal experiment (light at 15° C., dark at 38° C.), was not done.

Growth is erratic, some seedlings dying quickly, others putting forth several small but well-developed leaves. Media and conditions for sustained growth are not yet satisfactory. As seedlings (Fig. 1) are now readily available, this step is accessible to experiment.

Fig. 1. An axenic *Drosera intermedia* seedling at 1 month (× 4). Black ovals are ungerminated seeds.

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Spore Liberation and Dispersal of Coffee Rust *Hemileia vastatrix* B. et Br.

Nutman, Roberts and Bock[1] have produced evidence recently that *Hemileia vastatrix* uredospores are not liberated into the air from fructifications by wind alone at speeds up to 12·5 miles/hr. and only in small quantities if the supporting leaf flutters violently. They infer that contact of water with the pustules is the principal method of liberation.

However, investigations in the field made during rainstorms at the Coffee Research Station, Ruiru, Kenya, by me in 1956 had shown that wetting of the lower leaf surfaces was usually not very marked and was principally by upward splash from lower leaves. Rust lesions are confined to the lower surfaces and would be too infrequently hit for this to be the predominant method of spore liberation. Microscopic observations of lesions brought in from the field showed that spores were not dislodged by air currents alone to any marked degree. However, with plants grown in the still air of a greenhouse, a light tap on a rusted leaf produced a visible spore cloud. Recently, rust lesions resulting from the inoculation of a coffee plant kindly supplied by the Royal Botanic Gardens, Kew, and kept in a laboratory were found not to liberate spores under calm conditions, but abundant liberation occurred when they were tapped lightly. A lesion tapped at 2–3 week intervals liberated 366,100 spores during three months, and only some further 50,000 could be washed off at the end of this period.

Water droplets allowed to fall on leaves at points situated above fructifications, previously kept in calm conditions, produced abundant spore liberation; for example, ten 5-mm. diameter droplets dropped from a height of 4 ft. liberated 396,600 spores. Further, from such fructifications, currents of air at speeds estimated as less than 1 mile/hr. produced marked liberation. A current not greater than 2 miles/hr. deposited 23,440 spores on 2·5 cm.^{2} of sticky surface held 1 cm. behind a fructification. These observations contrast strongly with those of Nutman[1].

Thus, under field conditions, liberation of spores into the air is probably considerable, though in accord with their rate of fall[1], and as liberation is probably most marked during rainfall, the majority may well be deposited near their point of origin as indicated by Burdekin's[2] observations. The epidemic spread may thus approximate to that expected for a fungus disseminated by rain-splash.

In fructifications kept under calm conditions, stocks of spores are built up by the fascicles of hyphæ which erupt through each stoma forming short, irregular filaments several spores wide. Small mechanical impulses cause these to break off and fall away. Under field conditions, few spores will be ready for liberation at any one time and this may explain Nutman's[1] observations, since his experimental material was brought in from the field.

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Production of Symptoms of Barley Leaf-Spot Disease by Culture Filtrate of *Helminthosporium sativum*

IT has been reported by several investigators[1–4] that symptoms of some diseases could be reproduced by the action of the culture filtrate on the host plant, instead of using the infecting spores.

During 1957–58 a series of experiments were carried out at the Phytopathological Laboratory, Willie Commelin Scholten, Baarn, Holland, on the susceptibility of the different Dutch barley varieties, namely, Agio, Balder, Piroline and Wisa, to the leaf-spot disease caused by *Helminthosporium sativum* P. K. and B. Pathogenicity tests were carried out under glasshouse conditions where seedlings of the four barley varieties were sprayed with a standardized suspension of *Helminthosporium* spores. The typical brownish necrotic spots appeared on all seedlings. The most resistant variety, as indicated by the size of the necrotic spots, was Balder, followed by Agio, then Wisa, while Piroline was the most susceptible. The same experiments were repeated, but under field conditions, where identical results on mature barley plants were obtained.

It was thought interesting to study the response of these different barley varieties to the probable toxic action of *Helminthosporium* filtrate. Accordingly, the fungus was grown on modified Fries No. 3 medium[4] for 4 weeks at 25° C. The filtrate was passed through a glass filter to get rid of spores and tiny mycelial fragments. The filtrate was distributed into sample tubes. For each of the four barley varieties, four tubes were prepared in each of which three cut shoots were dipped. The cut shoots dipped in the filtrate were left afterwards for 48 hr. at room temperature ranging from 15° to 20° C. Brownish necrotic spots, similar to those induced by spore infection, were clear on the four varieties. In addition to the necrotic symptoms, cut shoots showed wilting and twisting, and they became

dark green in colour. Necrotic lesions, which appeared due to the toxic action of the filtrate, had almost the same size in the four varieties, denoting that the resistant, as well as the susceptible, varieties of barley responded similarly to the toxic action of *Helminthosporium* filtrate. It was also possible to reproduce symptoms of oat blight by using the culture filtrate of *Helminthosporium victoriae*[1]. Dilution of the filtrate with water 1 : 100 did not affect symptom reproduction, while filtrate diluted to 1 : 1,000 failed to reproduce symptoms on any of the barley varieties.

During 1958–1959 pathogenicity of the Dutch strain of *Helminthosporium sativum* to the Egyptian barley variety Baladi 39 and to the Sudanese variety Baladi was tested. The two varieties proved to be moderately susceptible to infections by *Helminthosporium* spores. The degree of susceptibility of both varieties was more or less comparable with that of the Dutch variety Agio. When cut shoots of the Egyptian variety Baladi 39 and the Sudanese variety Baladi were dipped in *Helminthosporium* filtrate, prepared exactly under the conditions mentioned, the shoots wilted, twisted and became darker in colour, but no necrotic symptoms could be detected at all. Hence, reproduction of natural symptoms on these African varieties was unsuccessful by using the filtrate of the Dutch strain of *Helminthosporium sativum*. Such differences between symptom production by the fungal pathogen and symptom reproduction by its filtrate emphasize the specificity of the host variety on which natural symptoms can be reproduced. Further work on this point in which African strains of *Helminthosporium* are involved, and on the nature of the toxins produced by the fungus, is in progress, and full details will be published later.

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Effect of Excess Supply of Heavy Metals on Barley during Germination, with Special Reference to Catalase and Peroxidase

The injurious effect of the excess supply of heavy metals on plant growth, among other factors, has been attributed to induced iron-deficiency[1–3]. Recently[4–7], it has been shown that heavy metals such as cobalt, nickel, copper, molybdenum and chromium, when supplied in excess, depress chlorophyll and catalase; in some instances peroxidase is also depressed. The repeated application of iron–ethylenediamine tetra-acetic acid to plants receiving the heavy metals mitigates the effect of heavy metals[6] on chlorophyll, catalase and peroxidase.

In order to test the effect of heavy metals on catalase and peroxidase before chlorophyll had developed, some preliminary investigations were made on barley (*Hordeum vulgare*) and 'kharbooza' (*Cucumis melo*) during germination. Contrary to the results obtained with adult plants, in seedlings the synthesis of catalase seemed to be stimulated at 1 m*M* concentration of cobalt. The investigation was extended to other heavy metals, and the effect of cobalt, nickel, molybdenum and chromium supply, each at 1, 5 and 25 m*M* concentrations, on barley during germination was studied. Each of the excess heavy-metal treatments was superimposed on a basal solution[6]—the full nutrient solution. The seeds were soaked in the appropriate nutrient solution for 12 hr. and then transferred to Petri dishes containing filter paper liberally moistened with the solution in which the seeds had been soaked. The Petri dishes were maintained in diffused light at room temperatures which varied between 19° and 23° C.

Table 1. Effect of the Excess Supply of Heavy Metals on Germination and Catalase and Peroxidase.
Results expressed as percentage increase (+) or decrease (−) over values obtained with seedlings given basal solution (ref. 6)

Treatment		Catalase		Peroxidase		Germination (length of first formed root)
		a	*b*	*a*	*b*	
Cobalt	1 m*M*	+44	+53	−20	−15	+21
	5 m*M*	+53	+33	−11	−22	−35
	25 m*M*	+61	−10	−39	−64	−92
Nickel	1 m*M*	+30	+24	−6	−10	−27
	5 m*M*	+147	+61	−21	−49	−77
	25 m*M*	+105	+24	−64	−78	−96
Molybdenum	1 m*M*	+2	+27	−7	+13	+1
	5 m*M*	+25	−25	−11	−47	−86
	25 m*M*	−22	−57	−57	−76	No emergence
Chromium	1 m*M*	+36	−3	−28	−49	−89
	5 m*M*	+152	+35	−47	−72	−96
	25 m*M*	+112	+26	−71	−76	No emergence

a, Expressed on fresh weight basis; *b*, expressed on per-seedling basis

At 1 m*M* supply germination was slightly stimulated by cobalt and depressed by nickel and chromium. At 5 m*M* and 25 m*M* concentrations germination was depressed by cobalt, nickel, molybdenum and chromium.

There was active synthesis of catalase during germination—eight-fold increase in 120 hr. from the soaking of the seeds in the basal solution. Excess supply of cobalt, nickel and chromium stimulated the synthesis of catalase, the effect being very marked at 5 and 25 m*M* supply each of nickel and chromium. No stimulation of the synthesis of catalase was found with molybdenum at 5 and 25 m*M* concentrations. In the germinating seeds, the main region for the synthesis of catalase was the growing embryo, that is, the developing root and shoot tissue. At 5 and 25 m*M* supply of nickel and chromium, although the formation of catalase-synthesizing tissue was severely restricted, yet the catalase expressed on the per seed basis was found to be higher than in seedlings raised without the excess supply of heavy metals.

As compared with catalase, peroxidase synthesis was slow—less than two-fold increase in 120 hr. from the soaking of seeds in the basal solution. Excess supply of heavy metals depressed peroxidase. Uptake of oxygen by the seedlings, as measured in a Warburg apparatus at 32° C., and total dehydrogenase activity, as measured by the reduction of triphenyl-tetrazolium chloride, were also depressed by the heavy metals, the effects being more pronounced in the growing embryo than in the rest of the seedling.

The uptake of heavy metals continued even when the germination was severely depressed and in general the heavy metals depressed the uptake of iron.

The stimulation of the synthesis of catalase by the excess supply of heavy metals would mean not only the increased synthesis of the protein moiety of catalase but also increased synthesis or at least availability of iron-porphyrin for the formation of catalase. This result cannot be explained in terms of the hypotheses advanced to date to explain the role of the excess supply of heavy metals in plants.

Detailed results of the investigations will be presented elsewhere.

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ENTOMOLOGY

An Abnormal Form of Female Rat Flea, *Xenopsylla cheopis* Roths.

Rothschild, in 1911, separated the three species of fleas, *X. cheopis*, *X. astia* and *X. brasiliensis* of the genus *Xenopsylla* found on rats. *X. cheopis* is widely distributed in the tropics and is the principal plague flea in India. The three species (♀♀) mentioned here can be easily identified by the shape of the spermatheca. Until now, it has been reported that *X. cheopis* has a single spermatheca[1-3] (Fig. 1).

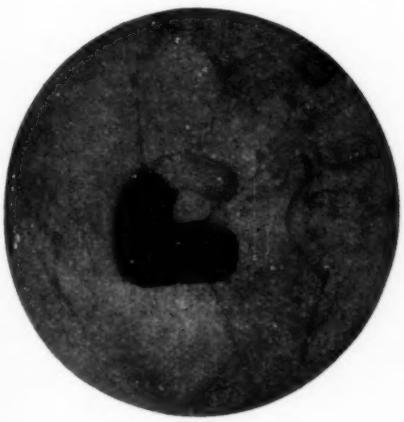

Fig. 1. (× 132)

During a routine collection of rat fleas from Delhi City for conducting insecticide susceptibility tests, a single specimen of *X. cheopis* (♀♀) was observed which has the normal typical spermatheca and another spermatheca-like structure (Fig. 2). The normal spermetheca has all the characteristics of a typical specimen: (1) the tail is much longer and at the base it is slightly wider than at the head; (2) the terminal part of the tail is longer and narrows gradually; and (3) the lower margin of the head and tail-end are at about the same level (Fig. 2, *A*). The additional spermatheca-like structure (the tail-end of which is just close to the terminal part of the tail of the typical form (Fig. 2, *B*) is pear-shaped and has a small blunt tail, but no distinct head portion.

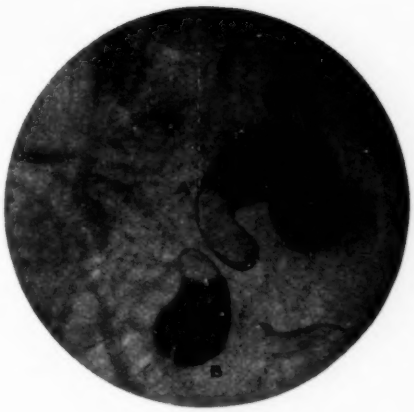

Fig. 2. (× 132)

There has been no previous record in the literature, so far as we know, of the occurrence of a similar abnormality in *X. cheopis* (♀♀).

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MICROBIOLOGY

Role of the Pentose Phosphate Pathway in *Pasteurella multocida*

It is acknowledged that in a large number of bacteria the main path for carbohydrate oxidation is the pentose phosphate cycle or other metabolic sequences not involving the Embden–Meyerhof pathway[1]. In the genus *Pasteurella*, work on *P. pestis* has shown that growing cells oxidize glucose through the pentose phosphate pathway[2], but resting cells oxidize glucose through the combined action of glycolysis and the citric acid cycle[3]. In contrast with this metabolic duality, in *P. multocida* the pentose phosphate cycle is the main path for carbohydrate oxidation, both in growing and resting cells, and in regard to the citric acid cycle its principal role seems to be to supply carbon skeletons for the synthesis of amino-acids. These conclusions are borne out by experiments carried out with *P. multocida* (strain Beaufort No. 28 from the Institut Pasteur cultured aerobically for 4 hr. (growing cells) or 24 hr. (resting cells)) at 37° and *p*H 7·2 in a medium made of meat extract, 5 gm.; yeast extract (Difco), 1 gm.; peptone, 30 gm.; sodium chloride, 5 gm.; glucose, 20 gm.; agar, 25 gm. and water up to 1 litre. Our observations can be summarized as follows.

(*a*) Resting *P. multocida* oxidizes glucose, fructose, gluconate and ribose. The Qo_2 values (μl. oxygen consumption/mgm. dried cells/hr.) of the cells incubated with 5 m*M* substrate, in m*M* phosphate buffer *p*H 7·2 at 37°, were 24 with glucose or fructose, 18 with gluconate and 12 with ribose (control, 2·3–2·6).

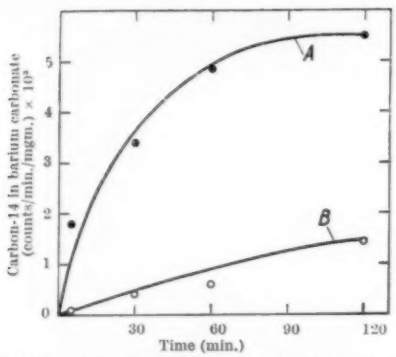

Fig. 1. Radioactivity of carbon dioxide evolved during the oxidation of [1-^{14}C] and [6-^{14}C] glucose by *P. multocida*. Incubation in double side-arm Warburg manometers. 23·2 mgm. (dry weight) of bacterial cells suspended in m*M* phosphate buffer, *p*H 7·2 with 5·3 m*M* [^{14}C] glucose (3·70 × 10^{6} counts/min.); total volume, 2·65 ml.; 0·15 ml. 2·7 *N* potassium hydroxide in the centre well and 0·20 ml. 3 *N* sulphuric acid in one side-arm; air in the gas space; temperature, 37°. At the intervals stated, the acid is tipped into the main compartment of the flask and the incubation continued for 15 min. The carbonate fixed in the alkali is precipitated as barium carbonate for carbon-14 counting as described previously (ref. 5). Line *A*, [1-^{14}C]-glucose; line *B*, [6-^{14}C] glucose. Qo_2, 22·2; control (without glucose), 1·3

(*b*) The specific activity of the carbon dioxide produced by oxidation of [1-^{14}C] glucose with resting cells of *P. multocida* is much higher than with [6-^{14}C]-glucose (Fig. 1). An approximate evaluation of the contribution of the pentose phosphate cycle to glucose oxidation can be calculated[4] by extrapolating to zero time the ratio of the specific activities of the carbon dioxide produced from [6-^{14}C] glucose and [1-^{14}C] glucose, respectively. From the results shown in Fig. 1, about 85 per cent glucose should be oxidized through the pentose phosphate cycle. With growing *P. multocida* the results are similar.

(*c*) After oxidation of carbon-14 glucose (1, 6 or *G*) by resting or proliferating *P. multocida*, the cells incorporate carbon-14. Paper chromatography and radioautography of methanol–water extracts[5] of the cells show the labelling of: (1) glucose, glucose-6-phosphate, fructose-1,6-diphosphate, 6-phosphogluconate, ribose-5-phosphate, and sedoheptulose-7-phosphate; (2) lactic, succinic, fumaric and malic acids; and (3) glutamic and aspartic acids, alanine, threonine, leucine, tyrosine and valine-tryptophan.

During the oxidation of [6-^{14}C] glucose by resting *P. multocida*, the kinetics of distribution of carbon-14 (per cent) in the sugar phosphates (and glucose) fit into the pattern of the shunt pathway. In fact, in the 0–3 min. interval, carbon-14 appears in the sugar phosphates in the order (1) glucose-6-phosphate, (2) 6-phosphogluconate and (3) ribose-5-phosphate, sedoheptulose-7-phosphate and fructose-1,6-diphosphate. After incubation for 3 min., variations in 6-phosphogluconate, ribose-5-phosphate and sedoheptulose-7-phosphate run closely parallel. On the other hand, the percentage of carbon-14 in glucose remains nearly constant. With [1-^{14}C] glucose as substrate, glucose-6-phosphate and 6-phosphogluconate again are labelled first, but the percentage of carbon-14 in the glucose reservoir decreases very rapidly. Similar kinetics are found with growing cells.

(*d*) In the presence of radioactive bicarbonate, gluconate oxidation promotes fixation of carbon-14 by resting *P. multocida*. It appears in glucose-6-phosphate, as would be expected from the reversibility of 6-phosphogluconate decarboxylation[6].

(*e*) Resting *P. multocida* oxidize pyruvate and the intermediates of the citric acid cycle. With substrate concentration 10 m*M*, the Qo_2 values (measured as in *a*) were 24 (pyruvate), 3·7 (citrate), 6·2 (DL-isocitrate), 19 (α-oxoglutarate), 26 (succinate), 13 (fumarate), 5 (L-malate) and 3·5 (control). After oxidation of [2-^{14}C] pyruvate, carbon-14 appears in the carbon dioxide evolved, the intermediates of the citric acid cycle and amino-acids. Growing cells incorporate more carbon-14 into the amino-acids (Table 1). It is to be noted that the labelling of cysteic acid shows that the bacterium is able to fix sulphur into organic substances.

Table 1. Distribution (per cent) of Carbon-14 in Amino-acids after Oxidation of [2-^{14}C] Pyruvate by *P. multocida*

Growing cells: 65 (initial) and 78 (final) mgm. *P. multocida* (dry weight) suspended in 22·4 ml. culture medium (see text) with 25·2 μmoles [2-^{14}C] pyruvate (17·76 × 10^{7} counts/min.).

Resting cells: 142 mgm. bacteria suspended in 10 ml. m*M* phosphate buffer, *p*H 7·2, with 112 μmoles [2-^{14}C] pyruvate (8·8 × 10^{7} counts/min.). Temperature, 37°. After 60 min. incubation, the suspensions were spun off and the cells suspended in 9 ml. methanol for paper chromatography, radioautography and carbon-14 counting (ref. 5).

Amino-acid	Carbon-14 fixed (percentage of total in the methanol–water soluble substances) Growing cells	Resting cells
Aspartic	0*	0·3
Glutamic	2·5	3·3
Alanine	5·6	0·4
Serine	2·1	0·3
Glycine	1·6	0·6
Threonine	6·6	0
Histidine	5·3	0
Lysine–Arginine	5·3	0·7
Leucine	2·8	0·2
Tryptophan–valine	6·0	0
Cystine	0	0·2
Cysteic	0	11·6

* Not detectable on the radioautogram.

In proliferating *P. pestis*, the shunt pathway provides pentose phosphates for nucleotide synthesis[7] and this can also be readily accepted in *P. multocida*; on the other hand, the pentose phosphate cycle also has a synthetic role in resting *P. multocida* as source of the pentose and heptose components of the polysaccharides of the bacteria[7].

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Spontaneous Development of *L* Forms of Streptococci requiring Secretions of Other Bacteria or Sulphydryl Compounds for Normal Growth

Certain strains of non-hæmolytic streptococci isolated in our laboratory from blood cultures of cases of subacute bacterial endocarditis and from otitis media, were found to require for their growth a substance secreted by most bacteria. They were isolated only due to the incidental presence of

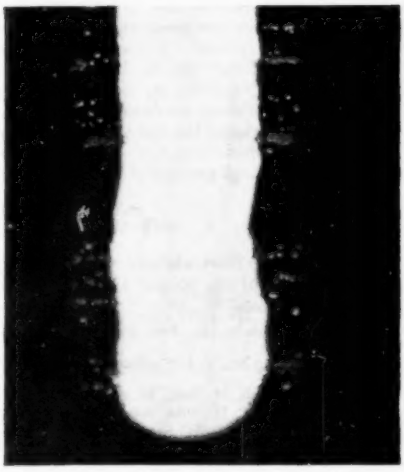

Fig. 1. Incomplete satellite growth zone of *Streptococcus* 6626. (Polarized light. × 5)

colonies of contaminating bacteria, around which they grew in satellite colonies. The bacterial secretion could be replaced by some sulphydryl compounds. In the absence of such substances, these strains tend to spontaneous development of *L* forms if an appropriate osmotic stabilizer is present in the agar medium. The sulphydryl compounds as well as the bacterial secretion seem to play a part in the formation of the cell wall of these streptococci.

The zone of satellite growth of the strains as shown on plates of *B.B.L.* soy trypticase agar or Difco heart infusion agar containing 5 per cent serum differed significantly from that known from *Haemophilus influenzae*, being much narrower and ending with a rather sharp edge (Fig. 1) which was accentuated if by incubating for a further 24 hr. the unused nutrients of the surrounding agar medium caused the outer colonies to grow in size.

A great variety of bacteria—Gram-positive and Gram-negative, catalase-positive and catalase-negative—including bacteroides, as well as actinomycetes and yeasts promoted growth.

The first substance found to be capable of substituting for the bacterial secretions was cysteine in concentrations of 0·5–1 mgm./ml. Its growth-promoting influence could not be explained by change of redox potential only, as anaerobic conditions did not alter the growth requirements of the strains.

Sodium thioglycollate, reduced glutathione and thiomalic acid were effective. Cystine, cysteic acid, methionine, sodium thiosulphate (up to 1 mgm./ml.) and elementary sulphur were not effective. It was therefore concluded that growth promotion by those substances depends on the sulphydryl groups.

The morphology of the streptococci was normal if growth was optimal. With lower concentrations of sulphydryl group substances, or at the outer limit of the satellite growth zone, the cocci showed extreme pleomorphism with swellings, global forms and elongations of different degrees. This suggested that the substance secreted by other bacteria, or the cysteine and like substances, had some function in the formation of cell walls, and that growth in *L* colonies might be produced without growth-promoting substances if support were given by adding to the medium substances known to have an osmotically stabilizing effect on bacterial cells the wall of which developed incompletely or not at all.

On plates of heart infusion agar + 5 per cent pooled human serum + 2 and 5 per cent sucrose respectively, two of the streptococcal strains grew in typical *L* colonies. The addition of sucrose to the medium had a remarkable influence also on the satellite growth of the streptococcus. Satellite colonies farther off were gradually diminished in size down to small transitional colonies, containing normal streptococcal forms together with swollen forms and net-like structures. Still farther off, beyond the influence of the bacterial secretion, there was pure growth of typical *L* colonies (Figs. 2 and 3). Many of these *L* forms remained stable even in the presence of other bacterial colonies or of cysteine.

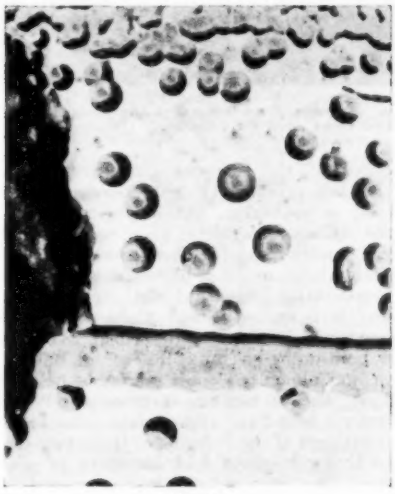

Fig. 2

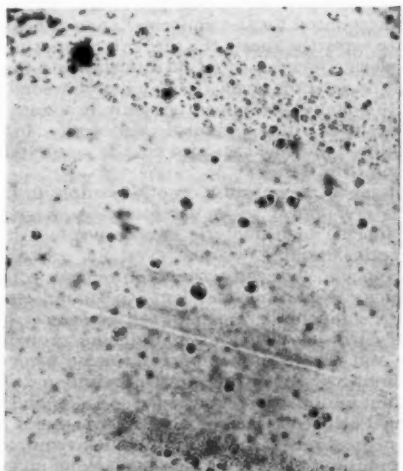

Fig. 3

Figs. 2 and 3. Development of normal satellite colonies (near growth of *E. coli* (Fig. 2) and of *L* colonies (beyond influence of bacterial secretion) (Fig. 3) from the same plating of *Streptococcus* 3554 on heart infusion agar containing 5 per cent human serum and 2 per cent sucrose. (× 35)

Fig. 4. 'Normalized' satellite growth zone of *Streptococcus* 6626 obtained under anaerobic conditions. (Polarized light. × 5)

Another of the streptococcal strains described here did not produce typical *L* colonies if sucrose was used as stabilizer, but in the presence of 1·5 per cent sodium chloride it grew colonies consisting of 'large bodies', swollen forms not stained by the Gram technique, and net-like structures.

A fourth strain also did not grow in typical *L* colonies, but on either of these media colonies developed which may be described as transitional stages containing a mixture of globular forms, swollen and elongated cocci stainable by the Gram technique, and of net-like structures. Possibly we have not yet found the appropriate osmotic stabilizer for the support of the *L* forms of these two strains.

Only living bacteria had an effect in promoting growth. The secreted substance passed through 'Cellophane'. It lost its growth-promoting property rapidly by oxidation. Obviously it was this lability which accounted for the abnormal appearance of the satellite growth zone, the growth-promoting substance losing its activity while diffusing into the more distant parts of the agar. Under anaerobic conditions, satellite colonies developed not only in a zone much broader than that obtained aerobically but also gradually diminished in size in the outer parts of the zone (Fig. 4).

Cysteine (1 mgm./ml.) or other sulphydryl compounds are known to be essential for growth from small inocula of hæmolytic streptococci[1]. Although our strains require cysteine even for growth from large inocula, basically the requirement might be the same. Nickerson and van Rij[2] found that sulphydryl compounds promoted cell division.

The effect of penicillin in inducing growth of *L* forms and the resistance of *L* forms to penicillin is well known. According to Dufrenoy and Pratt[3], penicillin inhibits the reduction of —S—S bonds of the bacterial cell constituents, whereas cysteine is known as a neutralizer of penicillin[4]. Nickerson[5] showed that a mutant of *Candida albicans* lacking an enzyme capable of reducing —S—S bonds of the cell wall grew in filaments instead of growing in the yeast form.

In our strains it may be assumed that we are dealing with an inability to reduce —S—S bonds required for the formation of the cell wall as a genetic metabolic defect, which is not necessarily lethal if appropriate stabilizers are present enabling them to continue life as *L* forms. There is an obvious analogy to the *L* form which Lederberg and St. Clair[6] succeeded in obtaining in sucrose agar from a mutant of *E. coli* deficient in diaminopimelic acid.

The possibility of using the specific growth requirement of these streptococci for development of a bioassay for sulphydryl groups is being studied.

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Specificity of the Mutagenic Action of the Alkylating Agents

Ethyl methanesulphonate is mutagenic when applied to mature bacteriophage particles in suspension[1]. Methyl methanesulphonate is either non-mutagenic or results in the induction of mutants at a frequency not much greater than the spontaneous frequency[2]. Krieg (quoted by Bautz and Freese[2]) has reported that ethyl methanesulphon te will not induce reversion of bacteriophage mutations induced by itself. Mutants induced by ethyl methanesulphonate can be induced to revert with 2-aminopurine[2]. Methyl methanesulphonate is much more lethal on a molar basis than is the ethyl derivative[1].

Freese accounts for the specificity of the ethyl derivative by supposing that both ethyl and methyl methanesulphonates combine with guanine residues within the deoxyribonucleic acid (DNA), but that only the ethylated guanine breaks down to form an apurinic acid at an appreciable rate. The apurinic acid is assumed to lead to errors in pairing, resulting in mutation during vegetative growth of phage.

Treatment of bacteriophage *T2* with ethyl methanesulphonate labilizes the phage, so that the number of plaque-forming units diminishes when the treated phage is stored in the absence of the alkylating agent[1]. It is implied by the Freese hypothesis (but is not essential to it) that this post-treatment decay in plaque-forming units represents the progressive depurination of the alkylated DNA.

I have found, in confirmation of earlier results[1], that treatment of bacteriophage *T2* with methyl methanesulphonate results in a post-treatment decay effect comparable to that induced by ethyl methanesulphonate. The degree of decay after treatment depends on the initial concentration of the alkylating agent used for treatment (Fig. 1). The methyl methanesulphonate effect occurs at a lower molarity but at a higher immediate inactivation than does the ethyl methanesulphonate effect. Treatment of bacteriophage with alkylating agents causes both an immediate and a delayed decrease in plaque-forming centres and may result in an appreciable frequency of induced mutation, depending on the agent. In this

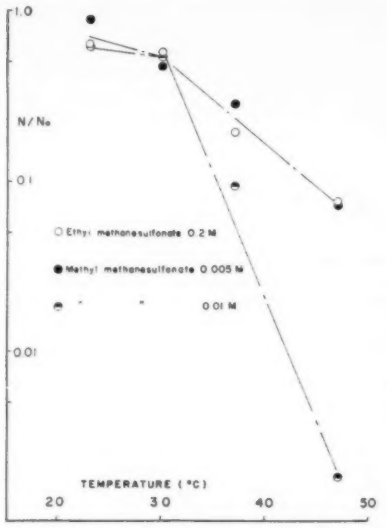

Fig. 1. Post-treatment decay of bacteriophage *T2* following treatment with alkylating agents.
Approximately 10^{12} phage per ml. were incubated in *M*-9 buffer with the indicated alkylating agent for 40 min. at 37° C. The survivals immediately following treatment were: non-treated, 1·0; 0·2 *M* ethyl methanesulphonate, 0·97; 0·005 M methyl methanesulphonate, 0·57; 0·01 *M* methyl methanesulphonate, 0·16. Following treatment, the suspension was diluted by a factor of 10^3 with *M*-9 buffer and samples were stored 21 hr. at the temperatures indicated. All values are corrected for the decay of non-treated material kept at the same temperature. Plaque-forming centres were determined by the agar layer method. The results are not plotted as an Arrhenius curve in order to indicate lack of a theoretical interpretation for the concentration effect

experiment the frequency of *r* plus mottled mutants in the material treated with ethyl methanesulphonate was about 7×10^{-3} immediately after treatment, 8×10^{-3} after 21 hr. at 30° C. and 6×10^{-3} after 21 hr. at 47° C. This frequency was five times the spontaneous. The number of mutants scored was so small that the frequency of mutants in the material treated with methyl methanesulphonate was not significantly different from the spontaneous frequency. In all parts of the experiment, 20 mutants per 19,000 total plaques were scored in the non-treated set, 89 per 17,000 plaques in the set treated with ethyl methanesulphonate, and 9 per 12,400 and 19 per 15,000 plaques in the sets treated with 0·005 *M* and 0·01 *M* methyl methanesulphonate, giving frequencies of $1·0$, $5·2$, $0·7$ and $1·3 \times 10^{-3}$ respectively.

Table 1. Effect of Methyl Methanesulphonate in inducing Reversions of *E. coli WP-2*

Treatment	Fraction surviving determined on: Minimal + tryptophan	Minimal + 2·5 per cent broth	Total revertants per ml.*	Revertants/10^8 survivors
None	1·0	1·0	60	13
Ethyl methanesulphonate (0·1 *M*)	0·87	0·87	3,120	780
Methyl methanesulphonate				
0·025 *M*	0·43	0·5	710	308
0·05 *M*	0·0005	0·0096	150	3,400

* Revertants were scored after 2 days incubation at 37° C. on plates of minimal medium agar supplemented with 2·5 per cent Difco nutrient broth (8 gm./l.). Treatment was for 30 min. in 0·1 *M*, *p*H 7·2, phosphate buffer. The cells were collected and washed from exponentially growing cultures in minimal medium plus tryptophan before treatment and then collected, washed and re-suspended in buffer following treatment.
† There were $4·6 \times 10^8$ viable organisms per ml. in the non-treated sample.

Methyl methanesulphonate is highly mutagenic for the tryptophan-requiring *WP-2* derivative of *E. coli* strain *B/r* (Table 1). An increased frequency of both large colonies (presumed revertants) and small colonies (presumed suppressors) results from treatment with this alkylating agent. The frequency is comparable with that induced by ethyl methanesulphonate. Methyl methanesulphonate causes an effect similar to that of ultra-violet irradiation of *B/r*, since treated bacteria give higher viability counts on medium supplemented with broth than on a synthetic medium[3]. There seems to be no post-treatment decay effect in bacteria comparable to that observed with bacteriophage[4]. It is known that in *Neurospora* dimethyl sulphate is even more effective as a mutagen at certain loci than is either diethyl sulphate or ethyl methanesulphonate[5].

I conclude, therefore, that the post-treatment decay reaction following treatment of bacteriophage with alkylating agents is not directly related to the mutagenic reaction, and that either the mechanism of the mutagenic action of alkylating agents differs in mature bacteriophage from that in bacteria, or that the specificity of the ethyl derivatives for bacteriophage mutagenesis is not completely explained.

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SOIL SCIENCE

Resistance of Humus to Decomposition

An organic soil (previously under wattle followed by grass) containing 8·51 per cent organic carbon (Walkley and Black[1], corrected) has been submitted 204 times to the following cycle: (*a*) moistened to field capacity; (*b*) allowed to decompose for 5 days at 25° C. in the macro-respirometer[2]; (*c*) oven-dried at 100° C. for 48 hr. After 105 such treatments it was calculated[3] that no further decomposition would occur, on moistening, after about the 250th treatment and that 73 per cent of the organic carbon originally present would have been mineralized. After 204 such treatments, however, the soil had become completely structureless and on moistening formed cohesive lumps, a condition adverse to aerobic decomposition. The experiment was therefore concluded and the soil analysed. A comparison of the results for the soil before and after the 204 treatments is given in Table 1.

The agreement between the calculated value for carbon mineralization after 250 treatments (73 per cent) and that found after 204 treatments (63·5 per cent) is satisfactory, particularly since the calculated value (based on the first 105 treatments) did not allow for the subsequent loss of structure during the later treatments, leading to decreased microbial activity and mineralization of organic carbon.

The main feature of interest is the high proportion of organic carbon and nitrogen that remained un-

XUM

Table 1

	Before	After	Mineralized (per cent)
Organic carbon (per cent)	8·51	3·11	63·5
Organic nitrogen (per cent)	0·69	0·37	46·4
C : N ratio	12·33	8·41	
*p*H (using 0·01 *M* calcium chloride)	6·0	7·9	
p.p.m. inorganic phosphorus (cold *N* sulphuric acid)	133	570	
p.p.m. organic phosphorus (Walker and Adams, ref. 4)	437	Nil	100·0

mineralized in spite of the enhanced microbial activity following the frequent moistenings of the soil after oven-drying. This is in marked contrast to organic phosphorus, which was completely mineralized. Thompson and Black[5], however, have shown that soil organic phosphorus is susceptible to hydrolysis with hot water. A duplicate soil sample was therefore kept at field capacity at 100° C. for 16 days (a period equivalent to that in which the experimental soil was in a hot moist state during its 204 treatments) when it was found that 53 per cent of the organic phosphorus and 7 per cent of the organic nitrogen were hydrolysed, with no increase on longer heating. With the experimental soil, therefore, the non-hydrolysable organic phosphorus compounds must have been microbially decomposed together with at least part of the hydrolysable compounds which are likely to be even more susceptible to microbial attack. It appears that potentially the whole of the soil organic phosphorus is fairly readily decomposable microbially. Since organic phosphorus complexes of soil are more readily extracted than other fractions of soil organic matter[6] they may also be more accessible to micro-organisms.

The increase in *p*H probably results from the release of bases during decomposition and the absorption of carbon dioxide by the sodium hydroxide in the respirometer vessel, which would prevent the formation of carbonic acid. The increase in ammonia amounted to only 1 m.equiv./100 gm. soil instead of the 23 m.equiv. demanded by theory. Presumably much of the ammonia was driven off under the hot, moist slightly alkaline conditions occurring early in the successive drying periods.

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PSYCHOLOGY

Effect of Attention in Peripheral Vision

The facts adduced by B. Babington Smith[1] appear to be new. At first it seemed that they might be explained in terms of what Stout calls "the conditions of attention"[2]—conditions that include change as well as extensity, intensity, duration, contrast and interest. If Stout is right, the factor missing in the object was change. I therefore repeated the experiment with objects containing moving parts, like the second-hand of a watch and an oscillating compass needle, and was amazed to find that the peripheral fade-out was, if anything, more marked.

The explanation of the phenomenon is, I believe, the following. When one inhibits the natural tendency to focus an object to which one is attending, one inevitably tends to reconstruct its details from memory. Though perception and memory are not inherently incompatible, the simplest kinds of image are, in fact, antagonistic to their sensational counterparts. Hence the effort to reconstruct the object from memory inhibits the exact perception of it. The most important inference to be drawn from the experiment is not that attention inhibits the perception of peripheral objects, but that the instinct of curiosity in its simpler forms operates by means of a commutative mechanism, whereby it automatically switches over to memory, when it is frustrated in perception. Experiments might be devised to show under what conditions the mind automatically switches back to perception, and, once the main facts had been elucidated, considerable improvements in methods of teaching and learning might be effected.

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[1] *Nature*, **189**, 776 (1961).
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In my communication of March 4, I spoke of placing objects on a table. As Mr. Thomson has deduced, the situation I described was static, and thus change, which is, broadly speaking, one of the essential conditions for perception, was lacking. Mr. Thomson's observations with the second-hand of a watch and a compass needle are very interesting but puzzle me, for I have failed to find what he describes. Others as well as myself have found that objects in motion do not disappear; though one correspondent has spoken of 'flicker', seen peripherally, disappearing when attended to.

The second part of Mr. Thomson's letter also puzzles me; while I can conceive that "the effort to reconstruct an object from memory inhibits the exact perception of it", it seems to me that switching over "by means of a commutative mechanism", as he suggests, ought to produce a memory image in place of the percept which disappears. This is not, however, what seems to happen.

Finally, Mr. Thomson writes that the facts I adduced appear to be new. Having failed to locate similar observations in Aubert[1], Baird[2] or Helmholtz[3], I had hoped this was so. Since publication, however, further search suggests that observations by Troxler[4], who was studying reports of a blind spot, and (according to Helmholtz's reference to their work) by Aubert and Förster[5], who were studying the sensitivity of the retina, refer to differential disappearance of objects in the peripheral field in a way conformable to what I have described.

Even nearer to the mark is a note by J. H. Hyslop[6], to which Prof. R. C. Oldfield has directed my attention, in which the following occurs: "When mentally preoccupied and having the eyes fixated on a given point or object I often notice the disappearance of a part of the indirect field of vision

I have tried to see whether it might not be due to the falling of the object on the blind spot, but uniformly discover that it is not, as the disappearing object may be on the side of the retina opposite the blind spot. On careful experiment and observation I find that the disappearance is directly related to the degree of abstraction, and that I can reproduce it artificially, if I am successful, as I sometimes am, in effecting the abstraction necessary and at the same time the proper adjustment of attention. It is difficult to produce the artificial abstraction required, but when I am successful I effect the disappearance of the object, which immediately reappears the moment attention is given to it without altering the fixation of the eyes. The effect seems to be that of making clear an actual impression, while attention in the previous experiment seems to destroy an after image."

Hyslop's use of the term 'abstraction' in contrast with 'attention' is interesting, and supports the view of several correspondents, particularly Dr. N. Moray of Sheffield, that the word 'attention' in my communication in *Nature* is a source of difficulty. It was of course with this possibility in mind that I had written "attend to one of the objects and try to see or describe it in detail".

Prof. O. L. Zangwill has suggested that the mechanism of disappearance is related to Hering's local adaptation, but while this may be so, I find no evidence of Hering having associated local adaptation with attention.

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[3] Helmholtz, H. von, *Physiological Optics*, 1924 ed., **2**, 42 and elsewhere (1866).
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Centrally Produced Geometrical Illusions

So little is known about the mechanism responsible for the well-known geometrical illusions that it is not possible to decide on general theoretical grounds whether they are the result of retinal processes, central processes or both. An experimental procedure to demonstrate that they are at least partly of central origin is therefore of interest.

The procedure consists of constructing a visual target, in which the illusion figure is seen in stereoscopic vision but not in monocular vision. The simplest way of achieving this is as follows. A sheet of glass is covered with an array of randomly placed dots. On a second glass sheet the well-known Müller–Lyer illusion, for example, is constructed out of dotted lines, the separation of the dots being about the same as those on the first sheet. The two are then mounted parallel to one another, separated by a few inches. When viewed monocularly under conditions which prevent head movements, shadows and other sources of monocular distance clues, the two planes are seen as merged and the Müller–Lyer figure cannot be distinguished. In binocular vision the two planes are easily separated and all subjects report the illusion.

The rigour of the experiment can be improved in two ways: (1) if the three vertices *A*, *B*, *C*, of the figure are marked by dots of a different colour, judgment of distance *AB* compared with distance *BC* can be made, and in fact turns out to be significantly different, under monocular and binocular conditions; (2) instead of using two real planes, the situation can be simulated by constructing stereoscope diagrams using an electronic computer[1]. This enables a rigorous control of factors such as dot density, and ensures that the signal received by each eye is a thoroughly pseudo-random array of dots; the information used by the stereoscopic mechanism is entirely contained in the relation between the two arrays.

A quantitative comparison of illusions produced in this way with their more familiar form involves intricate statistics and will be reported elsewhere. Figural after-effects can be produced under similar conditions, but are always weak, though I believe that this is due to incidental aspects of the procedure.

The work described here is part of the research programme of the National Physical Laboratory and this communication is published by permission of the Director of the Laboratory.

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[1] Julesz, B., *Proc. Fourth London Symp. on Information Theory*, edit. C. Cherry, 1961.

MISCELLANY

The National Reference Library of Science and Invention

The leading article in *Nature* of July 1 presents a timely challenge to all organizations concerned with the use of scientific and technical knowledge to think deeply about the plans for the National Reference Library of Science and Invention. This is certainly a project needing imaginative and sympathetic consideration, not only by librarians but also even more, perhaps, by the scientific societies and institutions and by technical interests in industry. It is earnestly to be hoped that, as representatives of potential users of the new library, they will study the plans and give their considered views soon, lest the preparatory work becomes too far advanced for them to be taken into account.

The success of the undertaking will, however, depend primarily on the degree to which the Government itself demonstrates its determination to treat the communication of research results, and technical knowledge generally, with the same seriousness and sense of urgency as are devoted to the acquisition of them. In this, the new Library could prove a most potent instrument. But on its two fundamental needs—adequate space to house the necessary documents and adequate staff to exploit them—Mr. Freeth's reply to the adjournment debate in the House of Commons on March 14 was anything but reassuring. The decision to split the holdings of the new Library between the British Museum site in Bloomsbury and the new building on the South Bank, while regrettable, is understandable. But it can only be regarded as a workable compromise on

two conditions: First, the South Bank building must be large enough to ensure that the bulk of the documents in frequent demand can be housed there, not only in 1965, but also for at least the following twenty years when, with the projected development of the Bloomsbury site, the situation might well be reviewed; and, secondly, arrangements must be made rapidly to transport or transmit documents held in the British Museum, so that the reader on the South Bank can have what he wants within minutes rather than hours. Much of the work of a reader in a reference library involves consulting many documents in conjunction with each other, and following up references found in them: delay in obtaining documents may make it impossible to complete a search and necessitate further visits. The obvious way to minimize this danger is to accommodate the greatest possible stock on the South Bank, and it is earnestly to be hoped that some way of increasing the space available will be found.

The Government seems to recognize that the new Library will ultimately need a staff which is well equipped professionally, particularly with scientists, and more numerous; but it is not enough to promise more staff when the Library becomes operational. In a lending library, records can give a reliable indication of users' habits; but virtually nothing is known as established fact about the use of reference material on open shelves. Surely now is the time to appoint a nucleus of the future staff, free of operational duties, to determine users' needs and to establish data on which detailed plans can be based.

Mr. E. M. Nicholson is doubtless wise to urge that now that a start has been made one should not jog the arm which lifts the cup. No one in his senses would wish to do so; the project must be given every chance to succeed. This means not only that critics should criticize constructively, but also that the Government should provide powerful support for the practical implications of its own proposals. Unless it does, the National Reference Library of Science and Invention may be little more than a less dingy Patent Office Library inconveniently and perhaps ineffectively linked with the British Museum.

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The leading article in *Nature* of July 1, on the National Reference Library and other issues, calls for comment in regard to the problems of scientific information. Miss Webb is certainly to be supported in her aim of recruiting graduate staff who will assist readers in exploiting the resources of the new Library. The National Lending Library, which will not be in such direct contact with its users, is in a much more difficult position to assist in information retrieval (so long as it remains primarily a library). It is not to be expected, however, that such national libraries should undertake large tasks of information retrieval for readers, and hence they are not the correct authorities to handle research on the problems of the collation and dissemination of scientific information. Furthermore, although the needs of users must, of course, be studied and so far as possible met, the organization of such libraries is best left in the hands of scientifically trained librarians, and should not be considered a matter for dictation by any body of users who are only too often unable to specify their questions clearly and are mostly unacquainted with the intricacies of such problems as classification and information techniques. In some respects, however, this position may be changing, since an increasing proportion of the users of the Patent Office Library comprises information officers.

The problems of information retrieval arise mainly, however, in special libraries and, more particularly, in information departments in industry, research associations, and the like. Many smaller (and perhaps even some larger) researches into information techniques are being carried out, with little publicity, in information departments of the larger industries, and steady progress has been made by the Classification Research Group; most of such groups are in regular contact with Aslib. The collation of such work and the promotion of new researches would, therefore, be far better undertaken by Aslib, with its new research department, than put under the vague ægis of the national libraries. The research being undertaken by Mr. Cleverdon on retrieval by different methods of classification, at the College of Aeronautics, Cranfield, under the auspices of Aslib (on a grant from the National Science Foundation, and not, be it noted, from the British Government) is an excellent example of pioneering research in this field. The Advisory Council on Scientific Policy does not appear to be adequately acquainted with the nature of the problems to be solved.

Mr. Nicholson is quoted on the need for "senior people trained professionally both as scientists and librarians" and reference is made to the "perennial discussions on training intelligence and information officers". I would point out that the Institute of Information Scientists, formed in 1958, is now concerned with education in information work and a two-year postgraduate evening course, based on the Institute's full syllabus, was started last January at the Northampton College of Advanced Technology, London. Examinations based on this syllabus, and leading to the granting of its certificate, will be held by the Institute. The provision of this course was arranged in consultation with Aslib. If sufficient students are forthcoming, it is hoped to start a repeat course as early as next September. It may also be of interest to mention that Dr. G. Malcolm Dyson, now research director to *Chemical Abstracts*, served as president of the Institute for three years, and this office has now been undertaken by Sir Lindor Brown, who is also president of Aslib. The training of information scientists (the science graduate plus information officer really needed by Mr. Nicholson) is thus being actively and constructively promoted.

Finally, it needs to be stressed that information techniques and the problems of information retrieval, vital to modern scientific work, are not just matters of empirical trial and error, either in library organization or with complex computers, but require steady theoretical and practical scientific investigation. For this, the subject must ultimately become a recognized branch of postgraduate studies at universities and technical colleges. Much greater Government support and encouragement are necessary, however, before this final stage of respectability is achieved.

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APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

SPECTROSCOPIST (preferably with a degree in physics or chemistry, and experience in spectroscopy) IN THE DEPARTMENT OF CHEMISTRY, to have general responsibility for the maintenance and use of a range of spectroscopic equipment in the Department, including N.M.R., infra-red, Raman and U.V., and to collaborate with members of the teaching staff on research problems, and supervise routine measurements required—The Registrar, The University, Manchester, 13 (August 21).

GORDON JACOBS FELLOW (with research qualifications) at the Royal Marsden Hospital for work in collaboration with the Institute of Cancer Research on the effects of ionizing radiations on nerves and the nervous system—The Secretary, Royal Marsden Hospital, Fulham Road, London, S.W.3 (August 25).

SUB-LIBRARIAN (graduate, with professional qualifications in librarianship) in the University Library—The Registrar and Secretary, The University, Bristol (August 25).

ASSISTANT IN ZOOLOGY IN THE DEPARTMENT OF NATURAL HISTORY—The Secretary, The University, Aberdeen (August 31).

ASSISTANT LECTURER OR RESEARCH ASSISTANT IN ENDOCRINE BIOCHEMISTRY, to assist in the analysis of steroids and other hormones as part of the research programme of the unit—The Registrar, The University, Liverpool, quoting Ref. CV/N (August 31).

ASSISTANT LIBRARIAN (with a good honours degree and preferably experience and professional diplomas)—The Librarian, University College of Sussex, Stanmer House, Stanmer, Brighton (August 31).

HORTICULTURAL ADVISORY OFFICERS (with a degree in horticulture, agriculture or science, and preferably practical experience in the production of horticultural crops and in field advisory and experimental work) IN THE AGRICULTURAL DEPARTMENT, New Zealand, to advise growers chiefly on fruit and vegetable production, and to conduct field experiments, surveys and special investigations—The High Commissioner for New Zealand, 415 Strand, London, W.C.2, quoting Ref. B13/2/4 (August 31).

LECTURER (with a university degree or equivalent qualification) IN THE DEPARTMENT OF TEXTILE CHEMISTRY—The Registrar, Manchester College of Science and Technology, Manchester, 1 (August 31).

SCIENTIFIC ASSISTANT, ABSTRACTOR (with a degree in agriculture or natural science, and able to read, or willing to learn, Russian)—The Director, Commonwealth Bureau of Pastures and Field Crops, Hurley, Berkshire (August 31).

SENIOR LECTURER (with a degree with honours in engineering, and preferably teaching experience) IN ENGINEERING; a SENIOR LECTURER with an honours degree in mathematics and postgraduate qualifications and experience in university teaching) IN MATHEMATICS; a LECTURER (with an honours degree in mathematics and preferably some postgraduate study) IN MATHEMATICS; and a LECTURER (with an honours degree with special interest in inorganic and/or physical chemistry, and preferably experience in university teaching) IN CHEMISTRY at the University College of Townsville, University of Queensland, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (August 31).

LECTURER OR ASSISTANT LECTURER (medically or non-medically qualified candidate) IN THE DEPARTMENT OF HISTOLOGY—The Registrar, The University, Liverpool, quoting Ref. CV/N (September 4).

GRADUATE RESEARCH ASSISTANT IN THE DEPARTMENT OF MECHANICAL ENGINEERING to work on problems of vibration and lubrication of machine tools—The Secretary and Registrar, The University, Southampton (September 9).

CHEMISTS and BACTERIOLOGISTS (with a degree in chemistry and bacteriology) IN THE AGRICULTURE DEPARTMENT, New Zealand, for the investigation, analysis and research into dairy products, raw materials and allied by-products—The High Commissioner for New Zealand, 415 Strand, London, W.C.2, quoting Ref. B13/2/A (September 15).

LECTURER/SENIOR LECTURER IN HORTICULTURE at the University of Sydney, Australia, to undertake teaching and research in fruit and vegetable production—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (Australia and London, September 15).

CHEMICAL ENGINEER, PHYSICAL CHEMIST or PHYSICIST (honours graduate with at least two years' research experience) in the Unit Operations Group, Chemical Engineering Section, C.S.I.R.O., Melbourne, to be engaged on either of the following projects: (a) a study of the dynamic characteristics and controllability of closed circuit grinding, and other solid-handling processes, using modern techniques, including frequency response; or (b) investigations within the general field of water desalination—Chief Scientific Liaison Officer, Australian Scientific Liaison Office, Africa House, Kingsway, London, W.C.2, quoting Appointment No. 608/14 (September 16).

PRINCIPAL PHYSICIST (with a first- or second-class honours degree in physics) in charge of the Department of Medical Physics—The House Governor, King's College Hospital, Denmark Hill, London, S.E.5 (September 16).

READER (preferably with a science degree or a degree in architecture, and relevant research experience) IN BUILDING SCIENCE in the Newcastle Division of the University of Durham—The Deputy Registrar, University Office, 1a Kensington Terrace, Newcastle-upon-Tyne, 2 (September 21).

SENIOR LECTURER (with a medical qualification and good experience in clinical biochemistry) IN CHEMICAL PATHOLOGY at the University of Otago, Dunedin, New Zealand—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1, or The Registrar, University of Otago (London and New Zealand, September 30).

READER IN HUMAN GENETICS IN THE DEPARTMENT OF ZOOLOGY, University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (October 15).

DIRECTOR OF THE POULTRY RESEARCH CENTRE, Edinburgh—The Secretary, Agricultural Research Council, Cunard Building, 15 Regent Street, London, S.W.1 (October 31).

LECTURER (with a degree in science or agricultural science, with some experience in research and teaching in plant pathology) IN BOTANY at the University of Melbourne, Australia—The Secretary, Association of Universities of the British Commonwealth, Marlborough House, Pall Mall, London, S.W.1 (October 31).

ASSISTANT BIOCHEMIST, Basic grade (preferably holding the A.R.I.C., the Grad.Mem.R.I.C. or B.Sc.(Hons.) in chemistry or biochemistry) in the Group Laboratory at Crumpsall Hospital—The Hospital Administrator, Crumpsall Hospital, Manchester 8.

ASSISTANT EXPERIMENTAL OFFICER (with a pass degree, preferably in zoology and chemistry) for biochemical research on fisheries problems—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berks, quoting Ref. 61/24.

ASSISTANT, Grade B (with qualifications and experience to teach mathematics for Diploma in Technology courses and for degree courses in engineering and general science) IN MATHEMATICS—The Registrar (Dept. F), Bradford Institute of Technology, Bradford 7.

ASSISTANT PHYSICIST (with at least an H.N.C. in physics, or equivalent qualification) on the staff of the Area Chief Chemist located in Birmingham, for duties involving interesting work on instrumentation in a modern laboratory, together with development and maintenance of instruments associated with gas making plant—The Industrial Relations Officer, West Midlands Gas Board, 6 Augustus Road, Edgbaston, Birmingham, 15.

ASSISTANT PROFESSOR (with a Ph.D. or equivalent, and preferably a background and experience in marine biology) OF BIOLOGY to give instruction primarily in animal physiology—The Head, Department of Biology, Memorial University of Newfoundland, St. John's, Newfoundland, Canada.

GRADUATE RESEARCH ASSISTANT (with an interest in organic chemistry) IN THE DEPARTMENT OF CHEMISTRY—The Secretary, Queen Elizabeth College (University of London), Campden Hill Road, London, W.8.

HISTOLOGIST (with an honours degree in physiology or zoology or equivalent qualification, and preferably previous experience in histological methods) IN THE PHYSIOLOGY DEPARTMENT to be in charge of the histological work of the department, and to collaborate in research mainly in the field of endocrinology—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, Berks, quoting Ref. No. 61/25.

LECTURER (preferably with experience in the field of infra-red spectroscopy) IN ORGANIC CHEMISTRY for work up to Dip.Tech. and Grad.R.I.C. Part II level—The Registrar, Bristol College of Science and Technology, Ashley Down, Bristol, 7, quoting Ref. No. CST/61/32.

LECTURER (well qualified, and preferably with both teaching and research experience) IN INORGANIC CHEMISTRY—The Academic Registrar, Loughborough College of Technology, Loughborough, quoting Ref. 21/2/AF.

LECTURER (with appropriate qualifications and experience of carrying out practical research work in the field of clinical psychology) IN PSYCHOLOGY—The Secretary, Institute of Psychiatry, The Maudsley Hospital, Denmark Hill, London, S.E.5.

PHYSICIST/PHYSICAL CHEMIST (with a university degree and some experience in the conduct of research) to undertake research in connexion with problems in food manufacture—The Director of Research, British Food Manufacturing Industries Research Association, Randalls Road, Leatherhead, Surrey, marking envelope "Private and Confidential".

POSTGRADUATE RESEARCH ASSISTANTS (with a degree in biochemistry, chemistry, physiology, physics or zoology) IN THE DEPARTMENT OF PHYSIOLOGY AND BIOCHEMISTRY—The Secretary and Registrar, The University, Southampton.

RADIOBIOLOGIST (with a knowledge of dosimetry, and preferably a background in entomology and/or invertebrate zoology) for research and development on biological effects of ionizing radiation for application in a long-range biological control programme—The Director, Instituto Venezolano de Investigaciones Cientificas, Apartado 1827, Caracas, Venezuela.

RESEARCH ASSISTANT (with an honours degree in botany) to collaborate in compiling a Flora of Turkey—Dr. P. H. Davis, University Department of Botany, Royal Botanic Gardens, Edinburgh, 3.

RESEARCH FELLOW (honours graduate with special qualifications in botany, biochemistry or microbiology, and preferably experience of radiochemical techniques) IN PLANT PHYSIOLOGY IN THE DEPARTMENT OF BIOLOGICAL SCIENCES, to undertake research in algal metabolism—The Registrar (Department F), Bradford Institute of Technology, Bradford 7.

RESEARCH STUDENTS; RESEARCH ASSISTANTS (with a good honours degree or Graduate Members of the Royal Institute of Chemistry) and POSTDOCTORAL FELLOWS in the Organic Chemistry Section of the Department of Chemistry for work concerned with (1) photochemical reactions of aromatic compounds, or (2) the chemistry of unsolvated organomagnesium halides—Dr. D. Bryce-Smith, The University, Reading.

SCIENTIFIC OFFICER, CHEMIST (not above 45 years of age, with a first- or second-class B.Sc. honours degree in chemistry, or A.R.I.C. or F.R.I.C., with postgraduate experience preferably in food chemistry and insecticide analysis) in the Federal Ministry of Commerce and Industry, Federal Government of Nigeria, to undertake chemical work pertaining to the deterioration and protection of raw foodstuffs during storage in Nigeria—The Recruitment Attaché, Nigeria High Commission, 9 Northumberland Avenue, London, W.C.2, quoting Ref. E/C3239/1.

SENIOR LABORATORY TECHNICIAN, Grade I (with appropriate qualifications and experience)—The Head Master, King Edward VI School, Southampton.

XUM

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Aspects of Co-existence. By Sir William Hayter. (Nineteenth Montague Burton Lecture on International Relations.) Pp. 18. (Leeds: The University, 1961.) 6*d*. [96

The Grassland Research Institute. Experiments in Progress No. 13: Annual Report for 1959–60. Pp. 124. (Hurley, near Maidenhead: The Grassland Research Institute, 1961.) 7*s*. 6*d*. [96

Department of Scientific and Industrial Research. Scientific Research in British Universities, 1960–61. Pp. xiii+513. (London: H.M. Stationery Office, 1961.) 32*s*. 6*d*. net. [96

National Union of Journalists. Freelance Directory, 1961. Pp. ii+86. (London: National Union of Journalists, 1961.) [96

John Innes Institute. Fifty-first Annual Report, 1960. Pp. 48. (Bayfordbury, Hertford: John Innes Institute, 1961.) 3*s*. [96

The Royal Institute of Chemistry. Register of Fellows and Associates, 1960, with a Supplementary List of Graduate Members. Pp. xx+643. (London: The Royal Institute of Chemistry, 1960.) [96

Department of Scientific and Industrial Research: Geological Survey and Museum. British Regional Geology: East Anglia and Adjoining Areas. By C. P. Chatwin. Fourth edition. Pp. vi+101+8 plates. (London: H.M. Stationery Office, 1961.) 6*s*. net. [96

Ministry of Agriculture, Fisheries and Food. Bulletin No. 123: Diseases of Vegetables. By Lawrence Ogilvie. Pp. v+101+12 plates. (London: H.M. Stationery Office, 1961.) 7*s*. 6*d*. net. [96

Department of Scientific and Industrial Research. Memoirs of the Geological Survey of Great Britain—England and Wales. Geology of the Country around Monmouth and Chepstow. (Explanation of Sheets 233 and 250.) By Dr. F. B. A. Welch and Dr. F. M. Trotter. With contributions by T. R. M. Lawrie, J. D. D. Smith and R. W. Elliot. Pp. viii+164+2 plates. (London: H.M. Stationery Office, 1961.) 22*s*. 6*d*. net. [96

British Society for the Promotion of Vegetable Research. Eleventh Annual Report, 1960 (Oct. 1959–Sept. 1960.) Pp. viii+72. (Wellesbourne, Warwick: British Society for the Promotion of Vegetable Research, 1961.) [126

Agricultural Research Council. A Bibliography of Farm Buildings Research, 1945–1958. Part 5: Buildings for the Drying and Storage of Grain. Pp. 43. (London: Agricultural Research Council, 1961.) 3*s*. [126

Ministry of Agriculture, Fisheries and Food. Domestic Food Consumption and Expenditure: 1959—Annual Report of the National Food Survey Committee. Pp. vi+142. (London: H.M. Stationery Office, 1961.) 8*s*. 6*d*. net. [126

The Carnegie United Kingdom Trust. 47th Annual Report, 1960. Pp. viii+98. (Dunfermline, Fife: The Carnegie United Kingdom Trust, 1961.) [126

Freshwater Biological Association. Twenty-ninth Annual Report for the year ended 31 March 1961, including Reports of the Council and of the Directors, and Accounts for 1960–61, with Lists of Officers, Council, Staff and Members. Pp. 88+1 plate. (Ambleside: Freshwater Biological Association, 1961.) 4*s*. [126

British Electrical and Allied Industries Research Association. Technical Report L/T395: Factors Influencing Intrinsic Electric Strength. Breakdown of Alkali Halide Crystals. By R. Cooper. Pp. 21+23 figures. (Leatherhead: British Electrical and Allied Industries Research Association, 1960.) 21*s*. [126

Institute of Fuel. Origin, Objects and Activities of the Institute. List of Officers, Council and Committees. Alphabetical Lists of Corporate Members (Chartered Fuel Technologists) and of various classes of Non-Corporate Members. Pp. 290+57. Careers in Fuel. Pp. 31 (3 plates) (London: Institute of Fuel, 1961.) [146

Department of Scientific and Industrial Research: Building Research Station. National Building Studies: Research Paper 34: A Study of Space and Water Heating in Local-Authority Flats, 1956–59. By J. B. Dick, J. H. Madge and C. N. Craig. Pp. vi+110. (London: H.M. Stationery Office, 1961.) 6*s*. net. [146

Women Engineers in the U.S.S.R. (A Study of Women Engineers in the U.S.S.R. carried out under the auspices of the Caroline Haslett Memorial Trust.) Pp. 28. (London: The Caroline Haslett Memorial Trust, 25 Foubert's Place, W.1, 1960.) [146

Department of Scientific and Industrial Research: Building Research Station. Factory Building Studies No. 8: Colouring in Factories. By H. L. Gloag. Pp. iv+19+9 plates. (London: H.M. Stationery Office, 1961.) 7*s*. 6*d*. net. [146

Tuberculosis in Animals: Proceedings of a Symposium held at the Zoological Society of London, May 24th, 1960. Edited by J. N. Ritchie and W. D. Macrae. (Symposia of the Zoological Society of London, No. 4). Pp. v+94. (London: Zoological Society of London, 1961.) 2*s*. 6*d*. [146

Other Countries

Panjab University, Lahore. Nutritive Value of Food-Stuffs and Planning of Satisfactory Diets in Pakistan. Part 1: Composition of Raw-Food-stuffs. By Dr. M. I. D. Chughtai and Dr. A. Waheed Khan. Pp. vi+34. (Lahore: Division of Biochemistry, Institute of Chemistry, Panjab University, 1960.) Rs. 1–8–0; 2*s*. 6*d*. [126

Regional Research Laboratory, Hyderabad. Annual Report, 1959–60. Pp. xiv+217. (Hyderabad: Regional Research Laboratory, 1961.) [126

Publications de l'Institut National pour l'Etude Agronomique du Congo. Série Technique No. 64: Les Buffles Domestiques d'Asie Importés au Congo—Observations Faites après sept Années de Multiplication. Par Dr. M. Mammerickx. Pp. 97+26 photographies. (Bruxelles: Institut National pour l'Etude Agronomique du Congo, 1961.) 180 francs. [126

Organization for European Economic Co-operation: Office for Scientific and Technical Personnel. New Thinking in School Mathematics. Pp. 246. (Paris: Organization for European Economic Co-operation, 1961.) 10 N.F.; 15*s*.; 2.50 dollars; 8.50 D.M. [126

Tropical Fish Culture Research Institute, Malacca. Report for year ended August 31st 1960. Pp. 28+4 plates. (Batu Berendam, Malacca: Tropical Fish Culture Research Institute, 1961.) [126

Annual Report for the year 1959 of the Museums Trustees of Kenya and of the Coryndon Memorial Museum, Nairobi. Pp. 24+1 plate. (Nairobi: Museums Trustees of Kenya and Coryndon Memorial Museum, 1961.) 1*s*. [126

Ministère de l'Agriculture de la Province de Québec. Le Conseil des Recherches Agricoles. Recherches Agronomiques: Sommaire des Résultats, 1958–1960. Pp. 32. (Québec: Ministère de l'Agriculture, 1960.) [126

Smithsonian Institution, Washington, D.C. Publication 4466: The Victorian American. By Anthony N. B. Garvan and Peter C. Welsh. (Lithographs from the Harry T. Peters "American on Stone" Collection.) Pp. 30. (Washington, D.C.: Smithsonian Institution, 1961.) [126

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Paper 60–22: Ground-Water Resources of Plum Coulee Area, Manitoba (Townships 1 to 6, Ranges 1 to 5 West of Principal Meridian.) By J. E. Charron. Pp. iii+81. 50 cents. Paper 60–23: Preliminary Report on Hydrogeology, Ottawa-Hull Area, Ontario and Quebec. By L. V. Brandon. Pp. ii+17. 50 cents. (Ottawa: Queen's Printer, 1961.) [126

Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 103 (April 1961): *Bacillus thuringiensis* Berliner: Über seine Biologie, Pathogenie und Anwendung in der Biologischen Schädlingsbekämpfung. Von Dr. A. Krieg. (In memorian Dr. Ernst Berliner 1880–1957.) Pp. 79. (Berlin-Dahlem: Biologischen Bundesanstalt für Lind- und Forstwirtschaft, 1961.) 12.40 D.M. [126

Canada: Department of Mines and Technical Surveys. Geological Survey of Canada. Bulletin 74: The Jurassic Faunas of the Canadian Arctic: Middle and Upper Jurassic Ammonites. By Hans Frebold. Pp. ix+43+21 plates. (Ottawa: Queen's Printer, 1961.) 2 dollars. [126

University of California Publications in Geological Sciences. Vol. 34, No. 7: Paleoecologic Molluscan Geography of the California Pleistocene. By James W. Valentine. Pp. iv+309–442. 2.50 dollars. Vol. 37, No. 3: Foraminifera from the Sacate Formation South of Refugio Pass, Santa Barbara County, California. By Gordon R. Hornaday. Pp. 165–232 (13 plates). 2 dollars. (Berkeley and Los Angeles: University of California Press; London: Cambridge University Press, 1961.) [126

Organization for European Economic Co-operation. Fertilizers in Europe: Production, Consumption, Prices and Trade—1961 Study, 1958–1961. Pp. 97. (Paris: Organization for European Economic Co-operation; London: H.M. Stationery Office, 1961. 6 N.F.; 9*s*.; 1.50 dollars. [126

Smithsonian Institution, Washington. Contributions from the Museum of History and Technology, Paper 16: Drug Supplies in the American Revolution. By George B. Griffenhagen. (United States National Museum Bulletin 225.) Pp. 109–133. (Washington, D.C.: Government Printing Office, 1961.) 25 cents. [126

United States Department of Commerce: Coast and Geodetic Survey. Publication 65–1, Part 14: Plane Coordinate Intersection Tables (2½-Minute), Kansas. Pp. iii+124. 65 cents. Publication 65–1, Part 20: Plane Coordinate Intersection Tables (2½-Minute): Michigan. Pp. iii+187. 1 dollar. (Washington, D.C.: Government Printing Office, 1961.) [126

Smithsonian Institution: Bureau of American Ethnology. Bulletin 180: Symposium on Cherokee and Iroquois Culture. Edited by William N. Fenton and John Gulick. Pp. vi+292. (Washington, D.C.: Government Printing Office, 1961.) 1.25 dollars. [146

World Health Organization. Technical Report Series. No. 198: Teacher Preparation for Health Education—Report of a Joint WHO/UNESCO Expert Committee. Pp. 19. 1 Swiss franc; 1*s*. 9*d*. 0.30 dollars. No. 210: Standardization of Methods for Conducting Microbic Sensitivity Tests—Second Report of the Expert Committee on Antibiotics. Pp. 24. 1 Swiss franc; 1*s*. 9*d*.; 0.30 dollars. No. 214: Molluscicides: Second Report of the Expert Committee on Bilharziasis. Pp. 50. 2 Swiss francs; 3*s*. 6*d*.; 0.60 dollars. No. 215: Planning of Public Health Services—Fourth Report of the Expert Committee on Public Health Administration. Pp. 48. 2 Swiss francs; 3*s*. 6*d*.; 0.60 dollars. European Standards for Drinking Water. Pp. 52. 2 Swiss francs; 3*s*. 6*d*.; 0.60 dollars. International Work in Health Statistics, 1948–1958. By H. S. Gear, Y. Biraud and S. Swaroop. Pp. 56. 2 Swiss francs; 3*s*. 6*d*.; 0.60 dollars. (Geneva: World Health Organization; London: H.M. Stationery Office, 1960 and 1961.) [146

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